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133

# Phytopathology

## An International Journal

## Official Organ of
## The American Phytopathological Society

CONTENTS

Aeciospore infection in Gymnoconia interstitialis by penetration of the cuticle ..... S. M. Pady 453

Chemical studies on the virus of tobacco mosaic III. Rates of inactivation at different hydrogen-ion concentrations. W. M. Stanley 475

Influence of phosphorus and potassium supply on host susceptibility to yellow tobacco mosaic infection ..... Ernest L. Spencer 493

Infección experimental del centeno de Petkus (Secale cereale v. vulgare), por las caries del trigo: Tilletia tritici y Tilletia levis Raimundo Nieves 503

Nomenclature of the tobacco downy mildew fungus. E. E. Clayton and John A. Stevenson 516

Phytopathological Note ..... 522

Comments on the article by A. Kozlowski on little-leaf or rosette of fruit trees in California. W. H. Chandler and D. R. Hoagland

Book Reviews ..... 523

Report of the twenty-sixth annual meeting of The American Phytopathological Society ..... 528

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**Official Organ of The American Phytopathological Society**

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PHYTOPATHOLOGY

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MAY, 1935

# AECIOSPORE INFECTION IN GYMNOCONIA INTERSTITIALIS BY PENETRATION OF THE CUTICLE[1]

S. M. PADY[2]

(Accepted for publication November 24, 1934)

It is commonly accepted as a fact that aeciospore germ tubes of the rusts normally enter their hosts through the stomatal opening. A survey of the literature reveals that no exceptions to this general rule are known. Recently, however, the writer discovered that when aeciospores of *Gymnoconia interstitialis* (Schl.) Lagerh., the orange rust of Rubus, were sown upon healthy leaves the germ tubes were able to penetrate the epidermis at any point, irrespective of the presence or absence of stomata. This was accomplished by means of enlarged spore-like bodies, the appressoria, formed at the ends of the germ tubes. Since this type of penetration heretofore has not been reported, detailed series of inoculation experiments were undertaken. This communication gives the results of these inoculation studies as well as a detailed description of penetration and the establishment of infection, and at the same time throws more light upon the host-parasite relationship in the rusts.

The first record of the method by which the germ tubes of aeciospores effect an entrance into their hosts is found in the classical researches of de Bary (9) in 1863. He inoculated leaves with aeciospores of many species and observed that the germ tubes always entered through the stomatal opening. Urediospore germ tubes behaved in a similar fashion. Even when inoculations were made on hosts other than their own there was no difference in the type of penetration. Gibson (25) performed similar infection experiments with 18 different species using urediospores in 12 species and aeciospores in the remainder. Her results are similar to those of de Bary. Clinton and McCormick (17) found germ tubes of aeciospores of *Cronartium ribicola* entering the stomata of leaves of *Ribes* sp. Zeller and Lund (39) observed that in *Phragmidium rubi-idaei* aeciospore and

[1] This work was done during the tenure of a National Research Fellowship in Botany at the New York Botanical Garden, New York.

[2] The writer wishes to acknowledge his indebtedness to Dr. B. O. Dodge and to Dr. R. A. Harper for their valuable advice and criticism during the course of the investigation.

urediospore infection always took place through the stomata. Ashworth (7) has recently reported a similar method of infection by the aeciospore germ tubes of *Coleosporium tussilaginis.*

Clinton (16) was the first to make inoculations with the long-cycle strain of Gymnoconia. Aeciospores were placed on leaves in Petri dishes on June 11, and on July 14 teliospores were observed. He concluded that "infection from the aecial spores of Gymnoconia takes place only through the stomates of the leaves and the telial stage results from this."

In 1923 Dodge (20) successfully inoculated cultivated blackberry varieties with Gymnoconia aeciospores obtained from *Rubus canadensis* and also obtained teliospores on the leaves of both blackberries and dewberries by sowing aeciospores from the black raspberry. This latter host was in turn infected with aeciospores from the blackberry. Dodge, in a later paper (21), while studying the development and distribution of the stomata on infected leaves, came to the conclusion that these leaves bore teliospores earlier and more abundantly than normal leaves, and that "the ease with which they may become infected is, at least in part, due to accessory dorsal stomata and freedom from tomentum." Aeciospores were sown on leaves, and after 2 days in a moist chamber, the leaves were dropped in Flemming's fixative. After fixation the chlorophyll was removed and they were mounted on a slide and examined. Dodge reported that "the germ tube grows along the surface until it comes into the immediate vicinity of a stoma, then, if necessary, the end turns sharply, broadens out and sends an infection tube through the opening."

The so-called "abnormal" germination, in which the contents of the spore and germ tube move into a "secondary body" or vesicle has been reported by many authors. These structures have been observed on the germ tubes of aeciospores by Tulasne (35) for *Aecidium violae* and *Roestelia cancellata,* by Sappin-Trouffy (31) for *Uromyces erythronii, Puccinia rubigo-vera, Roestelia lacerata* and *Peridermium pini,* and by Dodge (19) for *Gymnosporangium transformans.* Similar bodies have been noted on urediospore germ tubes of *Cronartium asclepiadum* (Tulasne (35)), *Puccinia graminis* (Plowright (28) and Sappin-Trouffy (31)), *P. iridis, P. fagopyri* (Barclay (8)), *P. Polygonii, Phragmidium rubi, Cronartium flaccidum* (Sappin-Trouffy (31)), and *Cronartium ribicola* (Spaulding (33)). In all cases the spores were sown on water and their behavior on leaves is unknown.

## PRELIMINARY STUDIES

In January the writer had a number of infected blackberry plants growing in the greenhouse. Germination tests were made on water and on 2 per cent agar as the aecia matured, as it was not known definitely whether

these plants bore the long-cycle or short-cycle strain. Although only long germ tubes were formed, it was decided to study their behavior on leaves, as it is known that the aeciospores of the short-cycle strain sometimes produce long germ tubes Dodge (22).

Aeciospores were dusted over the lower surface of freshly washed leaves, placed in Petri dishes and left on a desk in the laboratory. A little water was added to the plate to maintain the humidity necessary for germination and care was exercised to avoid contact with the leaf. Small portions of the leaves were examined from time to time. The pieces were prepared for examination by first putting them in 95 per cent alcohol and boiling them until they had lost their chlorophyll. They were then transferred to lactophenol and boiled until the leaves were cleared. A very small amount of cotton blue usually was added to the lactophenol.

At the end of 24 hours the aeciospores had germinated, but instead of long germ tubes being produced the entire contents had become concentrated in a rounded body at the end of a short germ tube. Although stomata were present in abundance, not a single germ tube could be found entering them. The next examination, 6 hours later, disclosed a short intracellular hyphal strand in the epidermis immediately beneath this terminal body, henceforth to be referred to as the appressorium. In view of the results of Clinton (16) and Dodge (21), who both state that infection takes place through the stomata, this behavior was somewhat surprising. It was decided, therefore, to make a series of inoculations in order to obtain information on the aeciospore infection of *Gymnoconia interstitialis*.

## INOCULATION EXPERIMENTS

To obtain a generous supply of aeciospores, infected plants from different localities in the vicinity of New York were brought into the greenhouse and potted. Since the infected plants form witches' brooms, it is possible to recognize them even before the leaves appear. From a clump of blackberries known to be free from the rust, several plants were simultaneously potted, at the Botanical Garden, to ensure a supply of known healthy leaves. Aecia began to appear in about 30 days from the time of the planting and, as soon as the pustules had broken open, the aeciospores were used for inoculations. All inoculations were made in Petri dishes, as described above. The leaves were examined periodically and kept alive as long as possible. The cultures could be kept for a limited period only, because the leaves were not supplied with nutriment. In one instance, that of March 16, 3 cultures were maintained for a period of 25 days. During late May and early June the warm weather made it very difficult to keep cultures more than from 10 to 14 days. This, however, was sufficient time in which to determine whether or not infection had become established, for it had

been found in earlier experiments that the mycelium was abundant by the end of the third day.

As both the long-cycle and short-cycle rusts occur on blackberries, it is not possible to tell at once which strain has been collected. Accordingly, when the aecia matured, germination tests were made with spores from all plants. Of the total number of infected wild blackberries brought into the greenhouse, approximately one half proved to be infected with the long-cycle strain of the organism, indicating, therefore, that this strain is of common occurrence about New York City. Healthy and diseased roots of black raspberry, *Rubus occidentalis,* were collected at Tuckahoe, N. Y. The long-cycle rust is the only strain known for this host.

The results of inoculations from the rust on blackberries and black raspberries are summarized in tables 1 and 2. In the tables there is a natural division between those rusts that were preseason, that is, those forced in the greenhouse, and those that matured normally. A plus (+) sign indicates that penetration took place through the epidermis by means of appressoria. A negative (−) sign signifies no germination. The number in brackets following the date of the experiment indicates the number of the particular plant from which the inoculum was obtained.

On April 9 the leaves of two twigs were inoculated by spraying the lower surfaces of those of one twig and the upper surfaces of those of the other with a spore suspension from a rusted blackberry. Both twigs were

TABLE 1.—*Inoculations of Gymnoconia interstitialis on blackberry*

| | Healthy leaves | | | Infected leaves | |
|---|---|---|---|---|---|
| | Blackberry | | Black raspberry | Blackberry | Black raspberry |
| | Upper surface | Lower surface | Upper surface only | | |
| Gymnoconia from greenhouse | +Mar. 16 (1)<br>+Mar. 20 (6)[a]<br>+Apr. 9 (3)<br>−Apr. 11 (3)<br>+Apr. 21 (27)<br>+May 9 (62) | +Mar. 12 (1)<br>+Apr. 9 (3)[b]<br>+Apr. 24 (27)<br>+May 10 (62) | +May 4 (27)<br>+May 10 (62) | +May 17 (62) | −May 17 (62) |

| | Blackberry | Dewberry |
|---|---|---|
| Gymnoconia from field | +May 20 (Bot. Garden, N. Y.)<br>+May 28 (West Virginia)<br>+June 5 (Hunters Is., N. Y.) | +June 6 (Hunters Is., N. Y.) |

+ = Successful penetration by appressoria.

− = Negative result (*i.e.* no germination).

[a] Seedlings of *Rubus canadensis.*

[b] Leaves on twigs.

kept in a large glass moist chamber. Aeciospores germinated readily and penetration took place from the appressoria at the ends of the germ tubes. This experiment confirmed earlier observations that penetration takes place as readily on the upper (stoma-free) surface as on the lower (stoma-bearing) surface.

The inoculations of the next series, April 21 and 24, were made to determine the effect of various external factors upon penetration. One set was left on the laboratory desk at room temperature; a second was similar but kept in the dark; a third on the cement floor; a fourth outside on the window sill; a fifth on a shelf in the darkened storeroom; a sixth in an oven at 25° C.; a seventh in the oven at 30° C.; and an eighth in the greenhouse, where the temperature was high in the daytime and low at night. The general result was that in all cases penetration took place. In the third set no germination had taken place and the leaf was completely dried up. There was no difference between those kept in the dark and those in the light. In the set kept outside where the temperature ranged from 6° to 18° C. during the course of the experiment, penetration was equally as good as in the case of the checks kept at room temperature. At 25°, germination was very slow and development was greatly retarded. There was no penetration at 30° and there were many irregularities and peculiarities in the germ tubes. Low temperatures apparently favor penetration and further development, while high temperatures retard germination and penetration and result in many irregularities. If the room temperature was high at the time of inoculation, the percentage of germination was usually lower.

A series of inoculations was made on infected leaves, the spores being dusted on the upper surface. The leaves bore the systemic mycelium and mature pycnia were abundant. As will be noted in the two columns at the right in table 1, penetration takes place readily on infected blackberries, and was independent of the stomata, which are abundant on the upper surface. In the series on black raspberries conditions were apparently not favorable for germination since spores from the same plant sown at the same time on blackberry leaves germinated readily.

When Gymnoconia matured in the field (see Tables) several series of inoculations were made using healthy blackberry leaves gathered at the same time. From table 1 it will be noted that the results were positive for all inoculations. In one experiment healthy dewberry leaves were substituted for those of the blackberry. Penetration was accomplished here also with equal facility.

In table 2 inoculations with aeciospores from black raspberry have been summarized. These results agree very closely with those in table 1 in that there is successful penetration of both the upper and lower surfaces

TABLE 2.—*Inoculations of Gymnoconia interstitialis on black raspberry (Rubus occidentalis)*

| | Healthy leaves | | | | Infected leaves |
|---|---|---|---|---|---|
| | Black raspberry | | Blackberry | | Black raspberry |
| | Upper surface | Lower surface | Upper surface | Lower surface | |
| Gymnoconia from greenhouse | + May 7 (57)<br>+ May 8 (58)<br>+ May 10 (57)<br>+ May 11 (49) | + May 14 (49) | − May 5 (57)<br>+ May 8 (57) | + May 7 (57)<br>+ May 14 (49) | + May 15 (49)<br>+ May 17 (49)[a]<br>+ May 19 (50)[a] |
| Gymnoconia from field | | | | | + June 14 Tuckahoe, N. Y. |

+ = Successful penetration.

− = Negative result (*i.e.* no germination).

[a] Leaves had infection only on one half of leaf.

of healthy and infected leaves of both blackberries and black raspberries. In the one negative case of May 5, there was no germination, probably because this set of Petri dishes had been accidentally left in the sunlight and the resulting high temperature had inhibited all germination.

As a final check, partially infected leaves were inoculated (May 17, May 19). Since the infected portion of the leaf will bear stomata on its upper surface while the remainder of the leaf will be stoma-free (21) the dusting of aeciospores over the entire leaf should prove conclusively whether or not the stomata play any rôle in the act of penetration. Careful examination of the leaves showed that penetration by means of appressoria occurs over the entire area of the leaf.

In all of these inoculations penetration was never found to occur through the stomata. Although occasionally a few very long germ tubes are formed on the leaf, as for instance when the cultures are kept at 30° C., their growth could not be correlated in any way with the presence of adjacent stomata. Several cases were recorded where the germ tubes grew over the actual openings of the stomata, yet did not enter. When spores were placed on the lower surface of healthy leaves and also on the upper surface of infected ones, there was ample opportunity for stomatal penetration. As shown in the above tables, penetration takes place just as readily on the upper as on the lower side, irrespective of whether the host be blackberry, black raspberry, or dewberry or whether it be infected or not.

## Details of Penetration

Within 24 hours from the time of inoculation the appressoria are fully formed. In shape they are, in general, similar to the aeciospore, *i.e.*,

globoid or ellipsoid, though they are slightly smaller (Fig. 1). The appressoria may be formed at the end of a long germ tube or, on the other hand, may be so close to the spore that it is almost impossible to find the germ tube. Their dense contents and thin wall are in striking contrast to the empty aeciospore with its thickened wall and verrucose markings. Their development is illustrated in Plate I, 1–3. From one of the germ pores in the spore a germ tube grows out and its tip begins to enlarge (Pl. I, 1). As the contents move toward the tip the aeciospore is gradually emptied and the appressorium becomes more and more dense (Pl. I, 1–3). A wall is laid down and the terminal appressorial cell is cut off (Pl. I, 3).

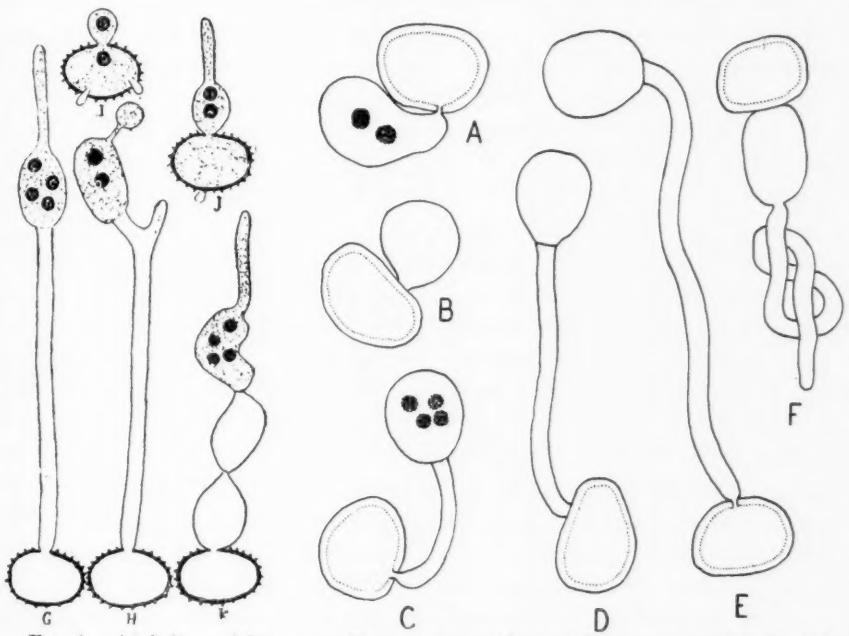


FIG. 1. At left, vesicles on aeciospore germ tubes of *Uromyces erythronii* (after Sappin-Trouffy (31) 1896). A–F. *Gymnoconia interstitialis*. A–D. Appressoria on leaves (24 hours). E. Appressorium formed at end of germ tube on agar. F. Appressorium that germinated but did not penetrate. ×560.

In the majority of cases the appressorium lies directly below the aeciospore, that is, between it and the surface of the leaf (Pl. I, 8, 9). This renders it very difficult to see their connection. Where the spore lies to one side, the connecting germ tube is readily observed (Pl. I, 4, 5, 7). Sometimes germ tubes reach a considerable length. This is especially true where there are hairs on the leaf. The spores are caught in the hairs and when germ tubes are formed they grow downward until they reach the leaf surface, where they form the appressoria. On the other hand, the

spores that fall directly on the leaf form appressoria immediately beneath them, or to one side, at the end of very short germ tubes.

The leaves of the black raspberry are strongly tomentose on the lower surface. Spores dusted on this surface fall among the hairs and long germ tubes are produced. The dense tomentum prevents one from readily following the entire length of the germ tubes, but if the leaf be well cleared in lactophenol, it may be turned over and examined from the upper surface. Where the germ tubes have reached the epidermis, appressoria can readily be found.

The two spore nuclei move out into the appressorium (Pl. I, 1) and sometimes divide, even before the septum has been laid down (Pl. I, 2). In this figure the position of the two pairs of nuclei suggests that conjugate division has just been completed. The mature appressorium is regularly 4-nucleate (Pl. I, 3, 4). At this stage they closely resemble the vesicles found by Sappin-Trouffy (31) on the aeciospore germ tubes of *Uromyces erythronii*. This similarity is at once apparent in figure 1 in which some of his illustrations have been reproduced for comparison with the appressoria of Gymnoconia (Fig. 1, A–F).

The appressorium flattens out against the epidermis (Fig. 2, A) and a penetration peg is formed. From above, the opening appears as a small white spot surrounded by a characteristic and slightly darker zone (Pl. I, 4, c). From this short penetration peg a penetration hypha is formed in the epidermal cell. Plate I, 4 shows a very young penetration hypha, while Plate I, 5 represents a slightly later stage. The cell contents pass in and the oil globules render it very distinct. Growth in the epidermis is rather limited, the hypha growing along the floor of the cell for a short distance; it then passes through the cell wall into the intercellular spaces below. This is well illustrated in figure 2, B, a cross section through an inoculated leaf. The aeciospore became detached during the preparation of the slide. The appressorium, however, remained attached to the leaf and the penetration hypha is a characteristic one.

The beginning of subepidermal or intercellular mycelium is clearly marked in the preparations made during the second and third days. In order to see this mycelium it is necessary to focus downward in the leaf tissue, indicating a change in level. This is best shown in cross section (Fig. 2). In plate I, 6, 53 hours after inoculation, a short hypha is seen growing away from the end of the penetration hypha and making its way deeper into the tissues, as indicated by the darker shading. The opening in the cell wall through which the contents pass is clearly seen in cross section (Fig. 2, B, C). The hypha in the latter figure has already branched and the two branches are following intercellular spaces in the palisade to the mesophyll below. This type of growth usually follows inoculation of

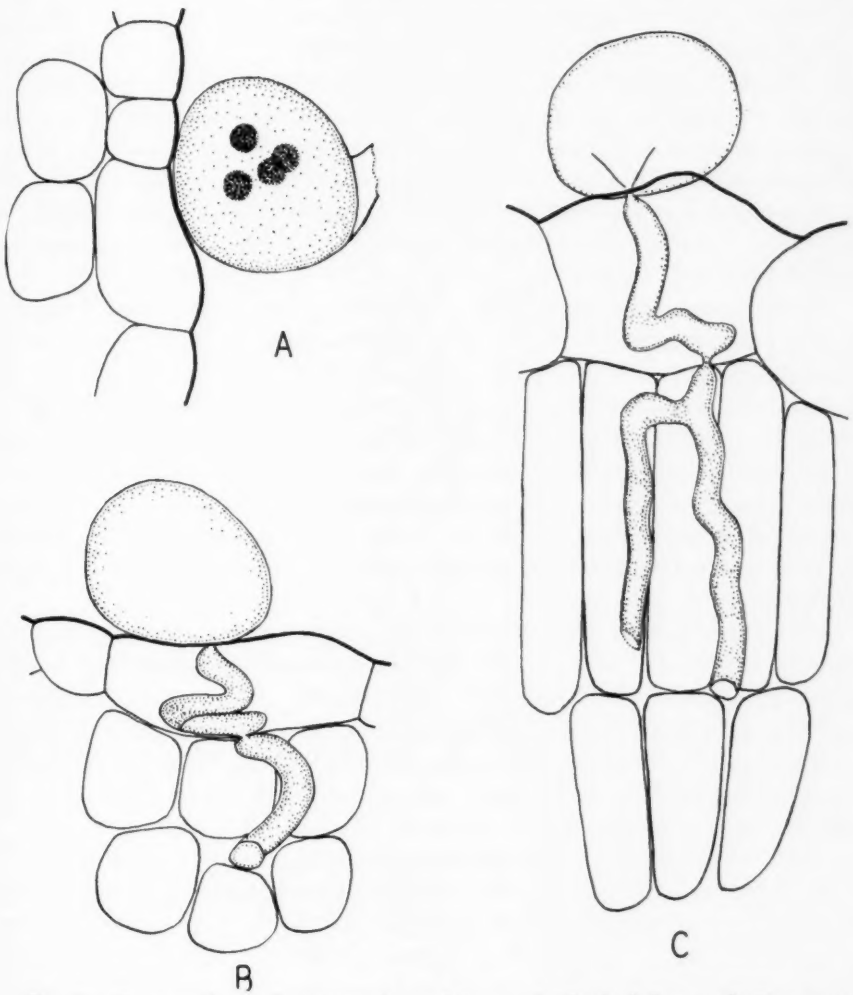


FIG. 2. Cross sections of appressoria on leaves. A. Typical four-nucleate appressorium. B. Penetration on lower epidermis. C. Well-advanced infection on upper epidermis. ×1240.

the upper surface. When the lower surface is inoculated the mycelium spreads near the leaf surface in the large intercellular spaces of the mesophyll.

Plate I, 7 is drawn from an inoculation on infected black raspberry. Although both the spore and the appressorium are partially lying over a stoma, it is at once apparent that penetration is entirely independent of them. The penetration hypha is short and the subepidermal hypha is dipping downward in the palisade tissue. In these infected leaves the haploid

mycelium is very abundant and haustoria are often present in the epidermal cell when the germinating aeciospore penetrates it (Pl. I, 8). Here, 53 hours after inoculation, the penetration hypha has branched and is giving rise to 2 hyphae in the intercellular spaces below. The hypha (f) at the right is growing toward the mesophyll between the palisade cells, faintly outlined in the drawing. The infected leaves were covered with pyncia and one case was observed where penetration had taken place directly on the base of the pycnium (Pl. II, 10). The host cell, already penetrated, contained 2 or 3 haustoria.

That the stomata play no rôle in penetration by germinating aeciospores is well illustrated in plate I, 9. The appressorium is typically beneath the empty aeciospore, and the penetration peg, 70 hours after inoculation, has entered the guard cell of a stoma. The penetration hypha is short and the subepidermal mycelium is extensive. There is a second hypha, which suggests that the appressorium has given rise to 2 penetration hyphae. It is quite possible, however, that this hypha may have come from a branch of the other subepidermal mycelium. The overlying guard cells, appressorium, and aeciospore make it very difficult to observe clearly the exact details.

The mycelium grows rapidly in the large intercellular spaces of the spongy mesophyll. Even at the end of the second day there may be considerable mycelium (Pl. II, 11). This usually is branched and the oil globules are very prominent. If a stoma is in the vicinity a hyphal branch usually enters the substomatal cavity (Pl. II, 12, 14). This is well-marked in infections of a week or more, the strands often running more or less directly from one substomatal cavity to another.

At the end of the fourth day the mycelium is fairly extensive (Pl. II, 13). There is no indication here of haustorium formation. The mycelium is beginning to outgrow the oil globules, and, while it is impossible to see nuclei along the course of the hyphae, at the tips the 2 nuclei of the dikaryon are readily observed. This figure also illustrates penetration at the extreme edge of the leaf. Many spores were found penetrating here, and the connection between the spore, appressorium, and penetration hypha may be clearly demonstrated. For the sake of clarity the cells of the leaf edge have been omitted from the drawing.

At the end of the fifth day haustoria begin to develop at various points on the mycelium. The epidermal cells are an excellent place in which to study their development, and all the drawings in figure 3, with the one exception of D, were taken from that part of the leaf. The haustorium mother cell usually is a short lateral branch of a main hypha (Fig. 3, E) and is regularly binucleate. If the mother cell is at the tip, the hyphal cell below continues to grow after the haustorium is formed (Fig. 3, F). The penetration peg is quite long and very slender (Fig. 3, A and B) and enters

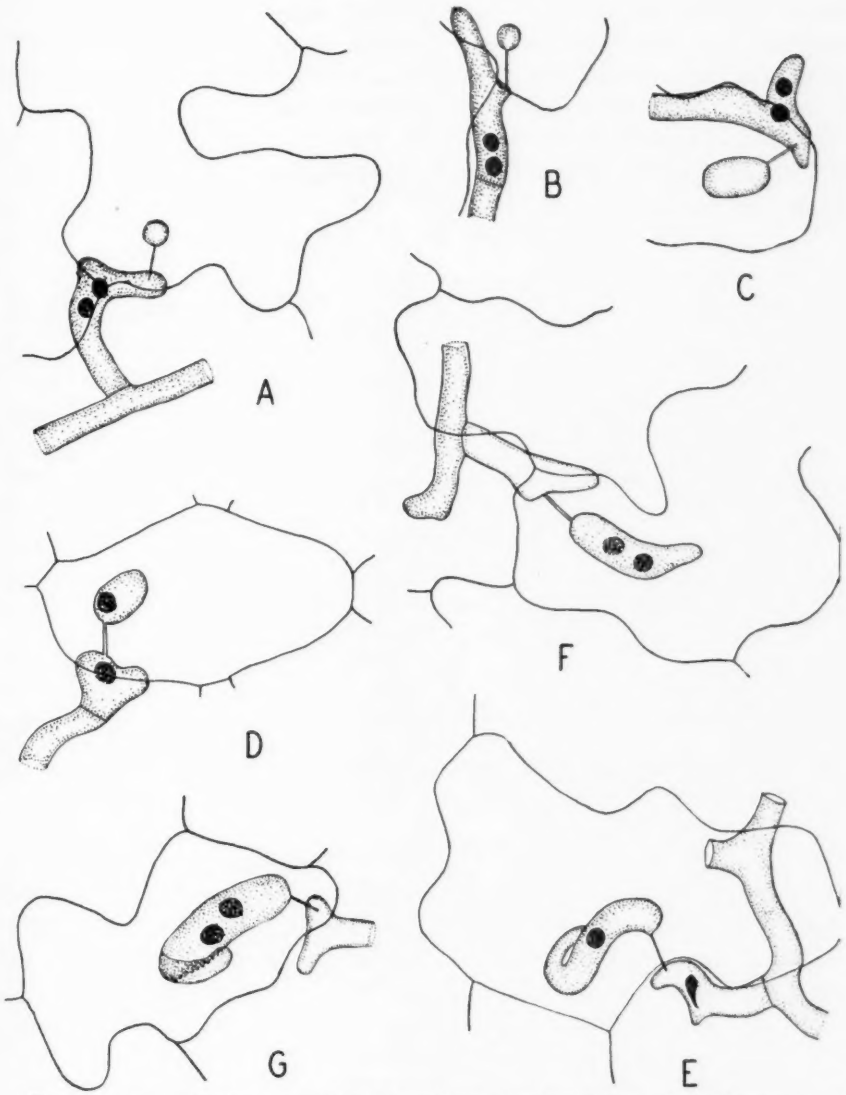


FIG. 3. A–G. Haustoria on diploid mycelium showing stages of development. All figures drawn from epidermal cells except D, which is in the mesophyll. ×1300.

the cell at a point near the side wall. At the tip a small round sac or vesicle appears (Fig. 3, A and B). This elongates as the contents of the mother cell move in (Fig. 3, C). One nucleus moves in (Fig. 3, D, E) and after a brief interval the other follows (Fig. 3, F). The mature haustorium is shown in figure 3, G. In older preparations it often is branched and the narrow tips lengthen, usually winding about the rest of the haustorium.

From this time on, the mycelium spreads rapidly through the leaf, par-

ticularly in the spongy mesophyll, forming haustoria in all tissues. The development of the mycelium, as well as the method of penetration, is well illustrated in the diagram in figure 4. Plate II, 14 was drawn from an 11-day-old preparation. Here, the mycelium was so abundant that only a small part of it could be included in the drawing. This case is remarkable in that no appressorium was formed, penetration taking place directly from the germ pore.

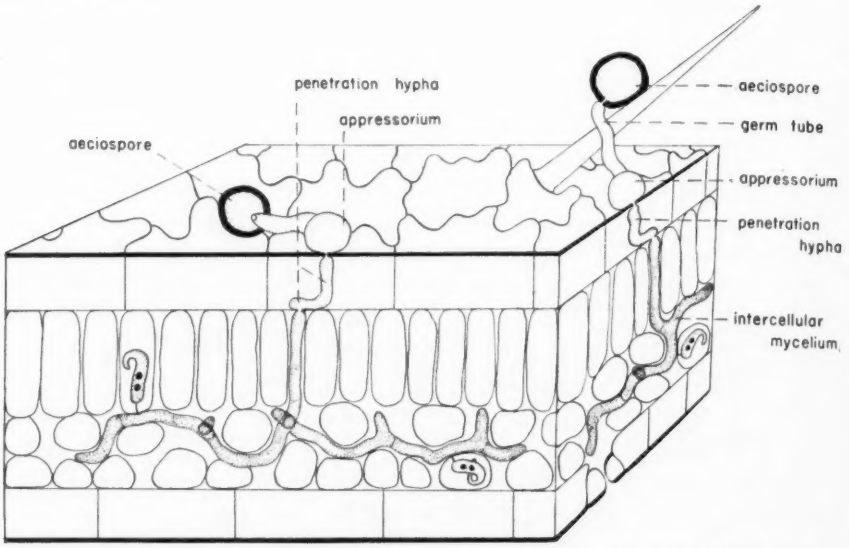


FIG. 4. Diagram of aeciospore infection in *Gymnoconia interstitialis*.

## Teliospore Production

At the end of 21 days teliospores were formed in the series of March 16 (Table 1). The leaves in this case were inoculated on the upper surface and the culture was kept for 25 days. By this time small black telia were abundant on the lower surface. Since these teliospores were produced in Petri-dish cultures under very artificial conditions, 2 seedlings were inoculated in the greenhouse as a check (Table 1). These seedlings were of *Rubus canadensis* and were about 3 inches high when inoculated. After being kept 4 days in a moist chamber they were placed on the greenhouse bench. Teliospores appeared on the 28th day on the lower leaves, none being found on the upper leaves. These results agree very well with those of Clinton (16) and of Dodge (20).

In the greenhouse, at this time, were several infected plants bearing both the long-cycle and short-cycle rusts. The short-cycle rusts had completed their sporulation period by the time the long-cycle ones had matured. About a month later the older leaves of the plants were found to bear teliospores, thus indicating that penetration takes place readily on the older

leaves under ordinary greenhouse conditions. In order to see whether or not infection takes place by appressoria in nature, field collections of leaves bearing Gymnoconia telia were examined. This material was collected by B. O. Dodge at Inlet, N. Y., on August 23. The basal leaves of the canes were thickly dotted with the telia (Fig. 5). When small portions of the leaves were cleared in lactophenol and examined, empty appressoria were found on the upper surface, and even in some cases the penetration hypha could be discerned. No appressoria were found on the lower surface.

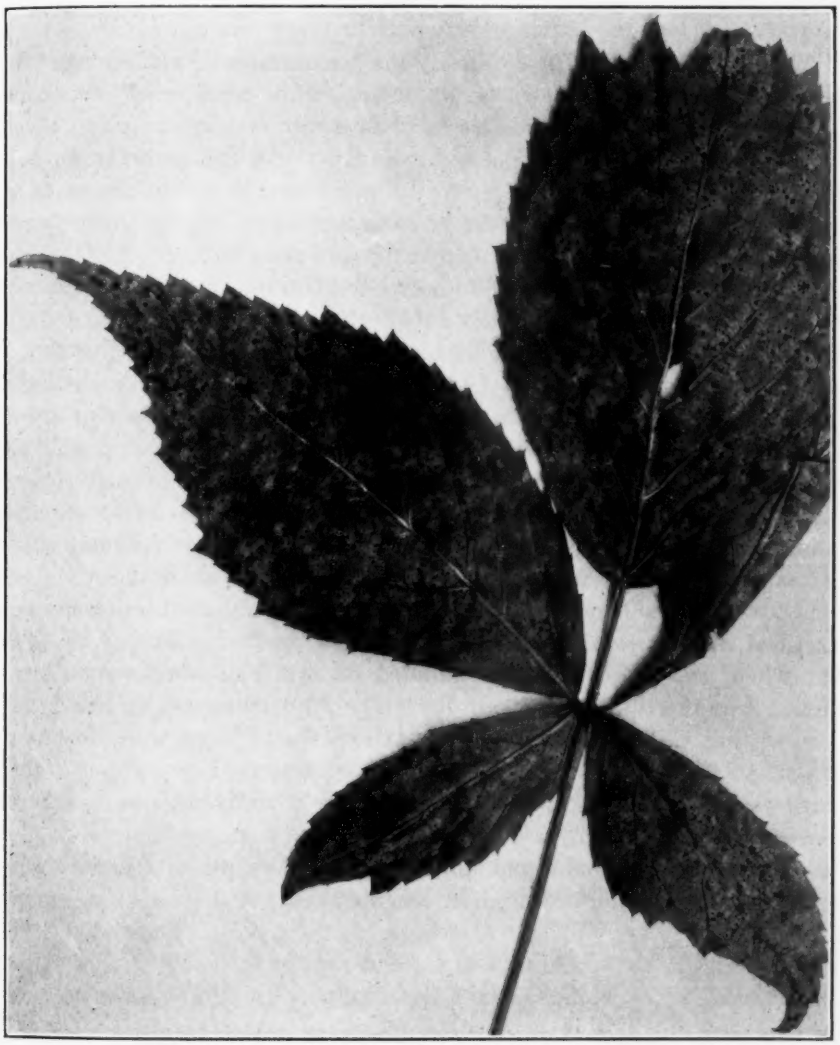

FIG. 5. Telial stage of *Gymnoconia interstitialis* on blackberry, showing localized infections.

In nature, wind-borne spores would fall in greater numbers on the upper surface than on the lower; and, while some infections would no doubt be from spores on the lower surface, the majority undoubtedly took place on the upper surface. In *Diplocarpon rosae* both Aronescu (5) and Dodge (23) have found that upper-surface inoculations gave rise to acervuli on the upper, while lower-surface inoculations produced fruiting bodies also on the lower surface. Gymnoconia, however, bears teliospores on the lower surface irrespective of the point of infection.

## DISCUSSION

Tulasne (35) was the first to study the germination of aeciospores. The spores of 9 species were sown on water and, while all formed germ tubes, 2 species, *Aecidium violae* and *Roestelia cancellata* (*Aecidium cancellatum*), produced oval or irregular vesicles at the tips. All the contents moved in and a second germ tube was formed. Similar vesicles were formed on the urediospore germ tubes of *Cronartium asclepiadum*. These were formed either at the end of long germ tubes or close to the spore.

Sappin-Trouffy (31) in 1896 found vesicles on the aeciospore germ tubes of *Uromyces erythronii, Puccinia rubigo-vera, Roestelia lacerata, Peridermium pini,* and also on the urediospore germ tubes of *Puccinia graminis, P. polygonii, Phragmidium rubi* and *Cronartium flaccidum*. According to Sappin-Trouffy, this last species germinated like Tulasne's *Cronartium asclepiadum*. The vesicles described by both Tulasne and Sappin-Trouffy greatly resemble the appressoria of Gymnoconia. The entire contents passed into the tip and Sappin-Trouffy also showed them to be regularly 4-nucleate. According to Sappin-Trouffy, they were never found on the host, and he believed that they were produced only when the germ tube grew out from a submerged spore. A somewhat similar situation has been described in *Gymnosporangium transformans* by Dodge (19). He found that when aeciospores were germinated on water a smaller pouch was formed near the tip of the germ tube. The 2 nuclei moved in and divided once, so that this body contained 4 nuclei. Dodge drew attention to the similarity between these structures and those described by Sappin-Trouffy. These terminal vesicles, found by the above investigators in 7 different species of rusts, probably represent appressoria; had they been formed on leaves, penetration would undoubtedly have taken place. Similar structures on the germ tubes of urediospores suggest that these also are appressoria.

Clinton (16) has stated that in *Gymnoconia interstitialis* infection takes place through the stomata, but the entrance of the germ tubes was not observed. Aeciospores were sown on leaves and a month later teliospores were formed. Details of penetration and the establishment of infection were not given and apparently Clinton assumed that, since the inocula-

tion was successful, the germ tubes had entered through the stomata. Clinton and McCormick (17) produced teliospores in Petri-dish cultures by sowing aeciospores on the lower surface, which bore stomata. Actual entrance of the germ tubes, however, was not observed.

The presence of accessory dorsal stomata on the leaves bearing the haploid systemic mycelium suggested to Dodge (21) that this might account for the presence of teliospores earlier on infected leaves than on healthy leaves. To obtain experimental evidence, aeciospores were dusted on leaves and 2 days later were placed in Flemming's fixative. After fixation the chlorophyll was removed, and the material examined *in toto*. Dodge observed that "the end . . . broadens out and sends an infection tube through the opening." The observation of these details would have been very difficult had not the material been adequately cleared following fixation. The end of the germ tube was observed to "broaden out" and from this broadened tip an infection tube was sent out. It is the writer's belief that this expanded terminal portion represents the appressorium and this is strengthened by the fact that an infection tube develops from it. Were the appressorium to lie directly over the opening, it would certainly seem that the infection hypha were entering through the stoma. As shown in plate I, 9, the infection tube or penetration hypha may enter the guard cells just as readily as any other part of the epidermis.

The production of teliospores earlier on infected leaves may be open to another interpretation. From inoculation experiments made on partially infected leaves (Table 2), it is clear that the infected area with its many accessory stomata possesses no advantage whatsoever over the adjacent healthy stoma-free areas. Nor does "freedom from tomentum" explain the ease of infection, since the lower, very tomentose surface of black-raspberry leaves was penetrated just as readily, the germ tubes growing down among the hairs and forming appressoria on the epidermis (May 14, Table 2). From earlier work (20) and from collections in nature, it is known that teliospores are formed first on the older basal leaves. Since Gymnoconia matures in the spring, healthy leaves—even the basal ones—are still young, while the infected leaves are more or less exhausted by intensive sporulation. They may be said to be "physiologically older" than the neighboring healthy leaves and, accordingly, readily infected and thus would bear teliospores much earlier.

Even when the aeciospores are germinated under different conditions, penetration always takes place by means of an appressorium. Absence of light does not retard penetration in any way. The only factor of importance is temperature. If the temperature rises the percentage of germination drops and various irregularities appear. In the inoculation experiments whenever the temperature in the laboratory was high the percentage of germination and of penetration was low. For instance, in the series of

May 10 (Table 2), 3 Petri dishes were used. In the first 2 the percentage of germination was low, while in the third there was no germination. As both Kunkel (27) and Clinton (16) have pointed out, temperature does not determine how the spores will germinate. In one or two cases long germ tubes are formed on the leaves without any appressoria. Since appressoria usually result through contact stimulus, it may be that this factor was lacking. Hasselbring (26) found that spores of *Gloeosporium fructigenum*, sown on convex drops of water, formed appressoria where they came in contact with the slide, while those that floated on the surface formed long germ tubes but no appressoria.

Penetration rarely occurs unless appressoria are formed. The aeciospore in (Pl. II, 14) is the only exception found. Bolley and Pritchard (13) claim that germ tubes of the urediospores of *Puccinia rubigovera* sometimes penetrated the cuticle without any appressorium. In corn rust, Weber (38) reported that they may or may not be formed. Boyle (14), Blackman and Welsford (12), Bensaude and Keitt (11), working on the other fungi, found little or no formation of appressoria. As Aronescu (5) has pointed out, these cases might indicate that it suffices to have contact between fungus and host.

Penetration of the cuticle apparently obtains in a manner similar to that resulting from germinating basidiospore infection as described by de Bary (10) and Waterhouse (37). A well-defined penetration peg is formed below the appressorium. This is very short and the penetration hypha thickens at a point very close to the cell wall. The literature on cuticular and cell-wall penetration has been adequately reviewed by Dey (18), Rice (29), Arthur (6) and recently by Aronescu (5), and need not be considered here.

An interesting feature of infection is the amount of mycelium developed in the leaf tissues before haustoria are formed. The first haustoria were found on the fifth day, by which time considerable mycelium had been produced. In a globoid spore of $20 \times 25$ μ diameter, the volume of cell contents is very large and thus capable of supporting a certain amount of mycelial development. On agar, germ tubes have been observed measuring over 700 μ long. It is to be expected, therefore, that that amount of mycelium could be produced before haustoria are necessary. By the time the first haustorium has been formed, however, the amount of mycelium is considerably greater. Some nourishment must have been obtained directly from the cells of the leaf by the hyphae as they grew through the intercellular spaces. Rice (29) found that corn rust (*Puccinia sorghi*) produced abundant mycelium before the first haustorium was formed, about the fifth day after inoculation. These results agree very closely with those of *Gymnoconia interstitialis*. In *Acanthostigma parasitica*, the causal agent of thread blight of tea, Butler (15) found that the fungus forms web-like films of hyphae over the lower surface of the leaf. There are no haustoria

or penetrating organs and the wefts of mycelium may be readily stripped from the leaf. From this he contends that the food may pass to the fungus through the unbroken walls of the cell.

Since the short-cycle rust becomes systemic following its attack of the young shoots, several inoculations with aeciospores of the long-cycle rust were attempted. If the diploid mycelium could enter the shoot it would be able to spread up the cane into the young leaves and down into the roots. Theoretically, this is possible, since stomata are unnecessary. The results of the inoculations, however, were negative. Germination occurred readily and appressoria were formed but no penetration took place. Inoculations on seedlings gave teliospores only on the older basal leaves, none being found on the very young leaves. Maturity of the tissues is apparently an important factor in the establishment of infection. Similar results were noted by Sheldon (32) in *Puccinia asparagi*. Seedlings of *Alium cepa* were completely immune, but when about 2 months old, were favorable for the development of aecia.

Although it generally is accepted that the entrance of aeciospore germ tubes is through the stomata (Arthur 6), an examination of the investigations made on this point reveals that comparatively few species have actually been studied. De Bary (9) made germination tests of both aeciospores and urediospores from 32 species. He was the first to observe their behavior when sown on the leaves of their hosts. In all cases the germ tubes were found to grow to the stomata and enter through the opening. Unfortunately, the number of species investigated in this fashion is not given, but, in a table recording the results of many cross-inoculations, 5 cases of aeciospore penetration are noted. In *Aecidium cyparissiae, A. tragopogonis, A. taraxaci, A. asperifolii* and *Uromyces appendiculatus* the germ tubes entered the stomata of not only their own hosts but also of other unrelated hosts. In these latter cases de Bary has pointed out that no infection took place. He came to the conclusion that in the rusts there are two methods of penetration. In one case the germ tube penetrates the cuticle. This takes place with the basidiospores developed from the promycelium. In the other case the germ tubes penetrate the host by means of the stomatal opening. This, de Bary concluded, is the method of aeciospore and urediospore penetration. Gibson (25) studied the behavior of 6 species on both their own and other hosts and found the aeciospore germ tubes always entered through the stomata. She concluded that this method of penetration was normal. Stomatal entrance has been observed by Clinton and McCormick (17) (*Cronartium ribicola*), and recently by Zeller and Lund (39) (*Phragmidium rubi-idaei*), and Ashworth (7) (*Coleosporium tussilaginis*). In these cases aeciospore infection through the stomata seems to be well established.

The emphasis of much of the recent work has been rather upon the

entrance of the urediospore germ tube. The researches of Ward (36), Evans (24), Stakman (34), Ruttle and Fraser (30), Allen (1, 2, 3, 4), Rice (29), and others have seemingly established the fact that infection from urediospores takes place through the stomatal openings. Since de Bary (9) and Gibson (25) concluded that the aeciospores and urediospores behave in the same way on their hosts it has been assumed that *all* aeciospores penetrate in the same way. When Clinton and McCormick (17) made their extensive series of Petri-dish inoculations they sowed aeciospores on those surfaces known to bear stomata. The successful infections obtained in 9 species were assumed to be the result of stomatal penetration. In order to make general statements as to the behavior of the aeciospore germ tubes, it is necessary to know the behavior of a large number of species. Until such data are available, it cannot be assumed that all species behave similarly. Our present knowledge of aeciospore germ-tube penetration is insufficient to justify the assumption that stomatal penetration is normal. Moreover, the discovery of appressoria on the germ tubes of the aeciospores of *Gymnoconia interstitialis* and their function in penetration of the cuticle, as well as the number of appressorium-like bodies described by many authors, suggests that stomata are not always necessary for successful infection from aeciospores.

## SUMMARY

The results of a series of inoculations with the aeciospores of the long-cycle rust, *Gymnoconia interstitialis*, show that infection takes place by means of appressoria that are formed at the end of the germ tubes. The entire contents of the aeciospore enter and the 2 nuclei divide once, so that the mature appressorium is 4-nucleate. A well-marked penetration peg passes through the cuticle and cell wall and a short penetration hypha is formed in the epidermal cell. The lower wall of the host cell is penetrated and the hypha enters the intercellular space just below. The mycelium spreads rapidly in the leaf tissue and haustoria may be observed after 5 days. Teliospores were formed from 21 days on. Penetration takes place equally well through the upper stoma-free surface and on the lower surface of leaves of either the blackberry, black raspberry, or dewberry. Leaves, already infected with the haploid stage, were penetrated in the same manner, the 2 mycelia not being antagonistic. Inoculations on partially infected leaves show that infection occurs just as readily on the healthy stoma-free area as on the infected area, which bears accessory stomata. Penetration may thus take place at any point on the leaf irrespective of the presence or absence of stomata.

## LITERATURE CITED

1. ALLEN, R. F. Cytological studies of infection of Baart, Kanred and Mindum wheat by *Puccinia graminis tritici* forms III and XIX. Jour. Agr. Res. 26: 571–604. 1924.

2. ALLEN, R. F. Cytological studies of forms 9, 21 and 27 of *Puccinia graminis tritici* on Khapli emmer. Jour. Agr. Res. **32**: 701–725. 1926.
3. ————. A cytological study of orange leaf rust, *Puccinia triticina* physiologic form 11, on Malakoff wheat. Jour. Agr. Res. **34**: 697–714. 1927.
4. ————. A cytological study of *Puccinia glumarum* on *Bromus marginatus* and *Triticum vulgare*. Jour. Agr. Res. **36**: 487–513. 1928.
5. ARONESCU, A. *Diplocarpon Rosae:* from spore germination to haustorium formation. Bull. Torrey Bot. Club **61**: 291–330. 1934.
6. ARTHUR, J. C. (in collaboration with F. D. KERN, C. R. ORTON, F. D. FROMME, H. S. JACKSON, E. B. MAINS and G. R. BISBY). The plant rusts (*Uredinales*). 446 pp. J. Wiley & Sons, Inc., New York. 1929.
7. ASHWORTH, D. Development and cytology of the uredo and teleutosorus on *Coleosporium tussilaginis*. La Cellule **43**: 189–200. 1934.
8. BARCLAY, A. A descriptive list of the Uredineae occurring in the neighborhood of Simla (Western Himalayas). Pt. III. Jour. Asiatic Soc. Bengal. **59**: 75–112. 1890.
9. BARY, A. DE. Recherches sur le développment de quelques champignons parasites. Ann. Sci. Nat. Bot. IV, **20**: 5–148. 1863.
10. ————. Comparative morphology and biology of the fungi, mycetozoa and bacteria. (Trans. by E. F. Garsney, revised by I. B. Balfour). 525 pp. Clarendon Press, Oxford. 1887.
11. BENSAUDE, M., and G. W. KEITT. Comparative studies of certain Cladosporium diseases on stone fruits. Phytopath. **18**: 313–329. 1928.
12. BLACKMAN, V. H., and E. J. WELSFORD. Studies in the physiology of parasitism. II. Infection by *Botrytis cinerea*. Ann. Bot. **30**: 389–398. 1916.
13. BOLLEY, H. L., and F. J. PRITCHARD. Rust problems: facts, observations and theories; possible means of control. Bull. N. Dak. Agr. Exp. Sta. **68**: 608–676. 1906.
14. BOYLE, C. Studies in the physiology of parasitism. VI. Infection by *Sclerotinia libertiana*. Ann. Bot. **35**: 336–347. 1921.
15. BUTLER, E. J. Fungi and disease in plants. 547 pp. Spink & Co., Calcutta and Simla. 1918.
16. CLINTON, G. P. New or unusual plant injuries and diseases. Bull. Conn. Agr. Exp. Sta. 222: 396–482. 1920.
17. ————, and FLORENCE A. MCCORMICK. Rust infection of leaves in Petri dishes. Bull. Conn. Agr. Exp. Sta. 260: 475–501. 1924.
18. DEY, P. K. Studies in the physiology of the appressorium of *Colletotrichum gloeosporioides*. Ann. Bot. **47**: 305–312. 1933.
19. DODGE, B. O. Studies in the genus Gymnosporangium. 1. Notes on the distribution of the mycelium, buffer cells, and the germination of the aecidiospore. Mem. Brooklyn Bot. Gard. **1**: 128–140. 1918.
20. ————. Systemic infections of Rubus with the orange rusts. Jour. Agr. Res. **25**: 209–242. 1923.
21. ————. Effect of the orange rusts of Rubus on the development and distribution of stomata. Jour. Agr. Res. **25**: 495–500. 1923.
22. ————. Uninucleated aecidiospores in *Caeoma nitens*, and associated phenomena. Jour. Agr. Res. **28**: 1045–1058. 1924.
23. ————. A further study of the morphology and life-history of the rose black spot fungus. Mycologia **23**: 446–462. 1931.
24. EVANS, I. B. POLE. The cereal rusts. 1. The development of their Uredo mycelia. Ann. Bot. **21**: 441–466. 1907.

25. GIBSON, C. M. Notes on infection experiments with various Uredineae. New Phytologist 3: 184–190. 1904.
26. HASSELBRING, H. The appressoria of the anthracnoses. Bot. Gaz. 42: 135–142. 1906.
27. KUNKEL, L. O. Further data on the orange rusts of Rubus. Jour. Agr. Res. 19: 501–512. 1920.
28. PLOWRIGHT, C. B. A monograph of the British *Uredineae* and *Ustilagineae*. 347 pp. K. Paul, Trench and Co., London. 1889.
29. RICE, M. A. The haustoria of certain rusts and the relation between host and pathogene. Bull. Torrey Bot. Club. 54: 63–153. 1927.
30. RUTTLE, MABEL L., and W. P. FRASER. A cytological study of *Puccinia coronata* Cda. on Banner and Cowra 35 oats. Univ. Calif. Publ. Bot. 14: 21–72. 1927.
31. SAPPIN-TROUFFY, P. Recherches histologiques sur la famille des Urédinées. Le Botaniste 5: 59–244. 1896.
32. SHELDON, J. L. The effect of different soils on the development of the carnation rust. Bot. Gaz. 40: 225–229. 1905.
33. SPAULDING, PERLEY. Investigations of the white pine blister rust. U. S. Dept. Agr. Bull. 957. 1922.
34. STAKMAN, E. C. A study in cereal rusts, physiological races. Bull. Minn. Agr. Exp. Sta. 138. 1914.
35. TULASNE, L. R. Second Mémoir sur les Urédinées et les Ustilaginées. Ann. Sci. Nat. Bot. IV, 2: 76–196. 1854.
36. WARD, H. M. Further observations on the brown rust of the bromes, *Puccinia dispersa* (Erikss.) and its adaptive parasitism. Ann. Mycol. 1: 132–151. 1903.
37. WATERHOUSE, W. L. Studies in the physiology of parasitism. VII. Infection of *Berberis vulgaris* by sporidia of *Puccinia graminis*. Ann. Bot. 35: 557–564. 1921.
38. WEBER, G. F. Studies on corn rust. Phytopath. 12: 89–97. 1922.
39. ZELLER, S. M., and LUND, W. T. Yellow rust of Rubus. Phytopath. 24: 257–265. 1934.

## EXPLANATION OF PLATES

All figures drawn with camera lucida from "in toto" preparations and shading is an attempt to represent the differences in level as one focuses downward. Darker parts are deeper in the leaf tissue.

Plate I. 1–2. Young appressoria at end of short germ tube. 3. Mature appressorium with four nuclei. 4. Penetration on midrib showing penetration peg (c) and young penetration hypha (d). 5. Typical penetration hypha (d) in epidermis (24 hrs.). 6. Young intercellular hypha (e) from penetration hypha (d). 7. Penetration on infected black-raspberry leaf. Hypha at f pushing downward in palisade. Note the accessory stomata. 8. Same as fig. 7. Penetration hypha (d) has branched in epidermis. A haustorium (g) from the systemic mycelium is in the same cell. 9. Penetration hypha (d) in guard cell. Intercellular mycelium (e) well advanced in mesophyll. a, aeciospore; b, appressorium; c, penetration peg; d, penetration hypha in epidermis; e, mycelium in intercellular spaces beneath epidermis; f, mycelium in palisade. ×750.

Plate II. 10. Penetration at base of pycnium (p) on infected black raspberry (24 hrs.). 11–12. Typical infections on lower epidermis (48 hrs.). 13. Penetration at leaf edge showing binucleate condition. Host cells omitted from drawing. 14. Penetration without an appressorium. Penetration peg (c) formed at germ pore (11 days). The explanation of the letters is given in legend for Plate I. ×750.

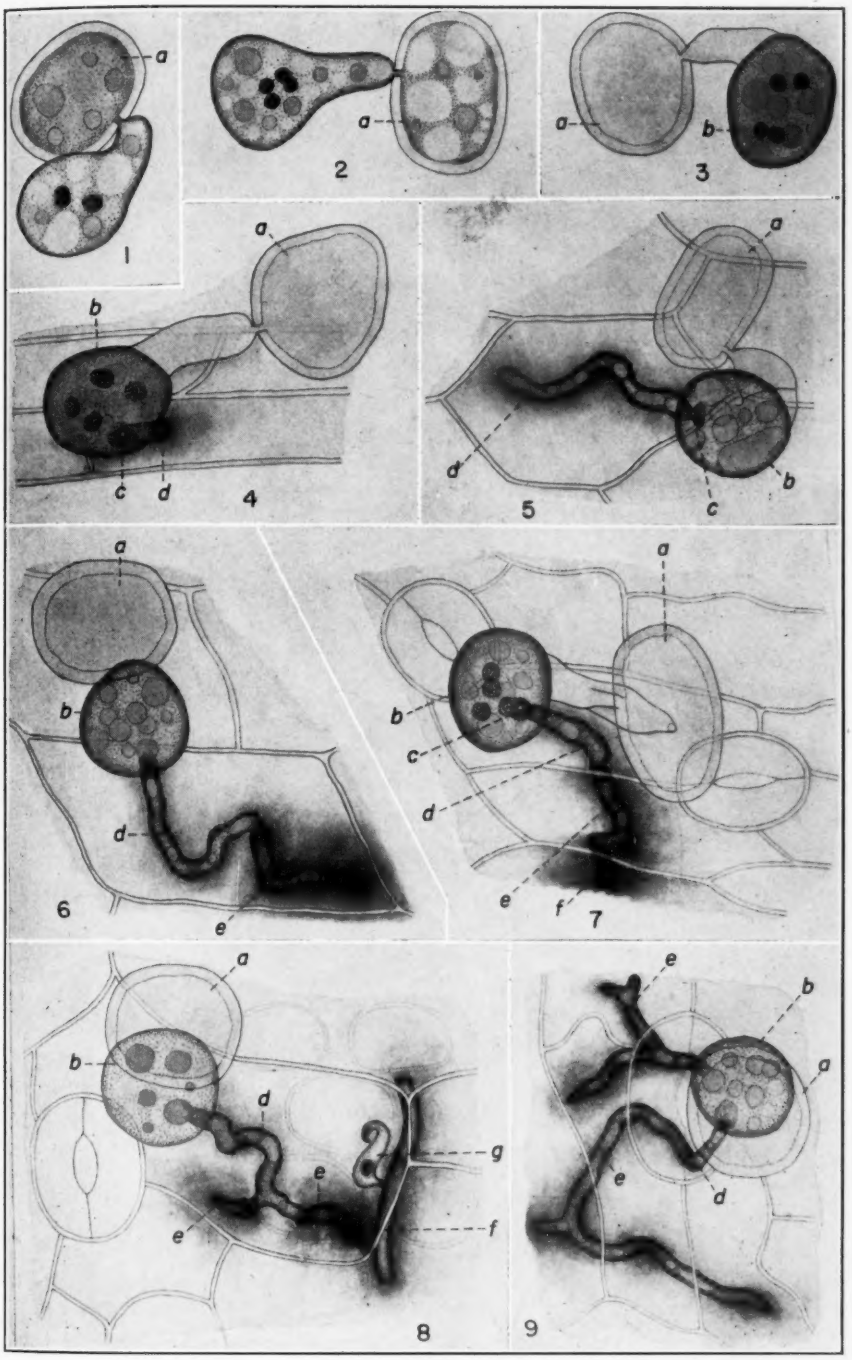


PLATE I

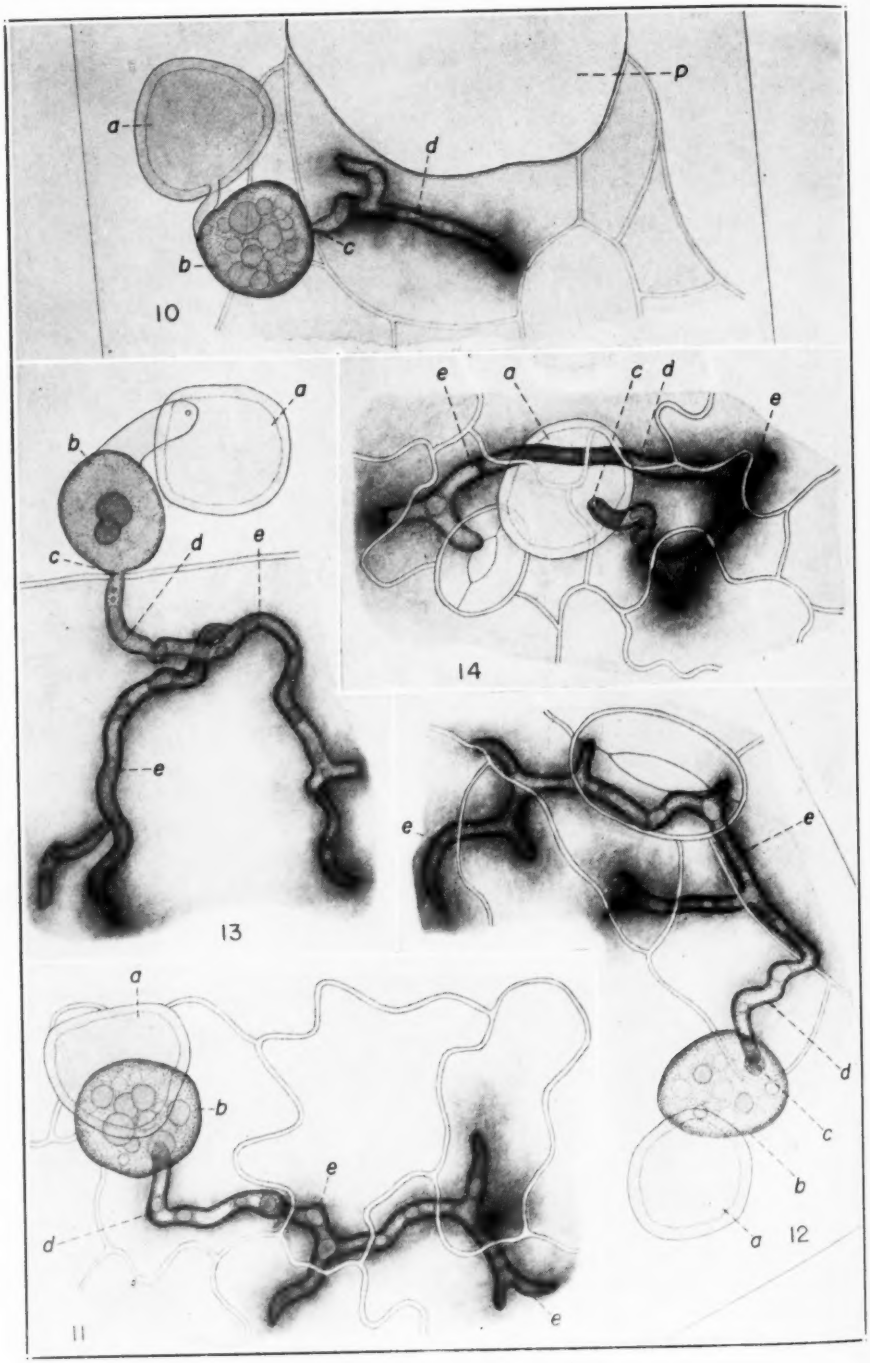


PLATE II

# CHEMICAL STUDIES ON THE VIRUS OF TOBACCO MOSAIC
## III. RATES OF INACTIVATION AT DIFFERENT HYDROGEN-ION CONCENTRATIONS

W. M. STANLEY

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### INTRODUCTION

A complete study of the chemical treatment of preparations of tobacco-mosaic virus necessitates knowledge of the range of hydrogen-ion concentration over which the virus is stable in order to distinguish inactivation due to excessive acidity or alkalinity from that due to other causes. In the concentration and purification of virus, the success of many precipitation, adsorption, and elution procedures, and of many chemical reactions, is dependent on the ability of virus to withstand certain hydrogen-ion concentrations. However, little is known of the effects of hydrogen-ion concentration on virus, there being no definite study on this subject and the few incidental observations being, in some instances, contradictory. One of the first reports in this connection was that of Allard (1) who, while engaged in a study of the effect of many chemicals on tobacco-mosaic virus, found that 1 per cent nitric or hydrochloric acid inactivated virus, but that 0.5 per cent acid left the virus infectious. Allard also reported that 0.1 per cent sodium hydroxide inactivated the virus, whereas it was not inactivated by 0.025 per cent sodium hydroxide. He did not measure the hydrogen-ion concentration of the solutions, and because of the differences in the buffering ability of different samples of plant juices it is impossible to translate his results to the pH scale. Nevertheless, Allard's findings indicate that excessive acidity or alkalinity inactivates virus and that alkaline reactions are probably more harmful than acid reactions. Brewer, Kraybill, Samson, and Gardner (3) reported that they found virus active at hydrogen-ion concentrations as high as pH 2.46, and that it was inactivated at pH 8.4 or pH 8.5, but, most strikingly, was reactivated when the concentration was brought to pH 7.74 or pH 5.33. This reactivation of virus has been neither disproved nor confirmed, although Vinson and Petre (16) found that complete inactivation did not occur in 24 hours at pH 10.5 and that the addition of acid did not restore the activity of solutions inactivated above pH 9.2. Fukushi (6) reported the virus to be active at pH 2 and pH 8.4, and to be most infectious between pH 4 and

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pH 7. Lojkin and Vinson (11) reported inactivation of virus at pH 3.3, but Vinson and Petre (16) found that other samples of virus retained their infectivity at pH 1.8.

The differences in the results obtained by different investigators are probably due to many factors, such as different methods of inoculation of virus, different test plants, different hydrogen-ion concentrations at the time of inoculation, different periods of time of exposure to the various hydrogen-ion concentrations, and to errors arising during the measurement of the hydrogen-ion concentration. Therefore, data available at present indicate only that virus is inactivated by excessive acidity or alkalinity, there being no complete data available regarding definite hydrogen-ion concentrations that either do or do not inactivate virus, and no data concerning the rates of inactivation of virus at different hydrogen-ion concentrations. As a preliminary to a chemical study on the virus of tobacco mosaic, it was necessary to secure these data. The results should be useful not only to chemists but to others working in the virus field, and they may serve to give an additional insight into the nature of viruses. The present paper records the results of a study of the rates of inactivation of tobacco-mosaic virus at different hydrogen-ion concentrations.

## MATERIALS AND METHODS

The virus used in the experiments here reported consisted of diluted untreated infectious juice and a purified virus preparation. The untreated infectious juice was obtained from plants of mosaic-diseased *Nicotiana tabacum* L. variety Turkish. The plants were cut and kept in a freezing room for a few days and then allowed to thaw just before pressing. The juice was filtered through 2 layers of bandage gauze and then diluted with 2 volumes of distilled water. The hydrogen-ion concentration of the juice was pH 5.4 before dilution and pH 5.7 after dilution. The purified virus was prepared from undiluted infectious juice by precipitation with lead acetate and elution with potassium phosphate, according to a modification of the method described by Vinson and Petre (16). This preparation was diluted with 9 parts of 0.1 M phosphate at pH 6.8. Then 1 per cent by weight of charcoal (Suchar 00) was added while stirring and the mixture was filtered through hardened filter paper 4 times. This purified preparation was colorless, water-clear, and somewhat more infectious than the diluted untreated infectious juice.

All determinations of hydrogen-ion concentration were made by means of a MacInnes type glass electrode (12) in order to eliminate errors due to foreign substances in the virus preparations. Unlike the hydrogen electrode, quinhydrone electrode, and color indicators, the glass electrode is unaffected by foreign substances such as electrode poisons, salts, reduc-

ing and oxidizing agents. The glass electrode is, therefore, especially suitable for the determination of hydrogen-ion concentrations of plant extracts. The apparatus used is similar to that described by Hill (7) and is capable of an accuracy of 0.001 pH unit.

In general, the experimental procedure used in the 3 sets of experiments to be reported consisted in adjusting separate portions of 250 cc. or more of the virus preparations to hydrogen-ion concentrations of from pH 0.5 to pH 12, in either 0.5 pH or 1.0 pH gradations, by the addition of acid or alkali. Immediate tests for virus infectivity at the various hydrogen-ion concentrations were made by the half-leaf method of inoculation, as described previously (9, 14, 15). After standing for various periods of time, portions of each of the preparations at the various hydrogen-ion concentrations were removed and adjusted to about pH 6 and tested against a control for virus infectivity. These tests were made by comparing the average number of lesions per half-leaf obtained on 5 or more half-leaves of *Nicotiana glutinosa* L. or 12 or more half-leaves of *Phaseolus vulgaris* L. variety Early Golden Cluster on inoculation with the various preparations, with the average number of lesions obtained on the other halves of the same leaves on inoculation with the standard or control virus preparation. In 2 experiments the virus preparations were kept frozen except during the tests for virus infectivity, but in a third experiment the preparations were kept at room temperature. Toluol was added to prevent bacterial action during the latter experiment.

In order to avoid using large volumes of acid or alkali and thus introducing an error due to dilution of the virus, solutions of 2.0 N HCl and NaOH were usually used to adjust the hydrogen-ion concentration. In certain instances concentrated HCl or a saturated solution of NaOH were used. The volume of the virus preparation was in no instance changed more than 7 per cent by the addition of acid or alkali. The error introduced by dilution of the virus to this extent is very small and may be neglected. In order to eliminate errors due to inactivation of virus by local excessive concentration of acid or alkali, the HCl or NaOH was added dropwise while stirring the virus preparation vigorously with a mechanical stirrer.

## EXPERIMENTAL

### Rates of Inactivation of Virus in Diluted Untreated Juice Prepared from Frozen Plants

Plants of *Nicotiana tabacum* variety Turkish were infected with typical tobacco-mosaic virus, when about 3 or 4 inches high, and grown in a greenhouse. When they had reached the stage of maturity indicated by the beginning of flower-bud formation, they were cut and placed in

a room held at −14° C. for a few days. The frozen material was allowed to thaw and the juice pressed out and filtered through 2 layers of bandage gauze. Two liters of filtered juice were diluted with 4 liters of distilled water. Twelve portions of 300 cc. or more of this diluted untreated infectious juice were then taken to hydrogen-ion concentrations of from pH 1 to pH 12 in gradations of 1 pH, by the addition of 2 N NaOH or HCl. In one instance, concentrated HCl was used in order to reduce the volume of the added acid. The amount of diluted untreated infectious juice used for each hydrogen-ion concentration is listed in the first column of table 1. The amount of acid or alkali needed to adjust each preparation is given in the second column, and the hydrogen-ion concentration of the adjusted preparation is given in the third column. It may be seen that the addition of acid or alkali did not change the volume of the preparations by more than 5 per cent, and that the hydrogen-ion concentrations were adjusted to within 0.05 pH of the desired values. Immediately after adjusting each preparation to the desired hydrogen-ion concentration, a small portion was removed and tested, at that hydrogen-ion concentration, for virus infectivity by the whole-leaf method on *Phaseolus vulgaris*. Additional 50 cc. portions of each of the preparations were removed and, after standing for 1 hour at 20° C., were readjusted to pH $6.0 \pm 0.2$ and then tested for virus infectivity against the control, using the half-leaf method of inoculation on *P. vulgaris* and *Nicotiana glutinosa*. The amount of 2 N NaOH or HCl necessary to adjust 50 cc. of each of the preparations to pH $6.0 \pm 0.2$ is given in the last column of table 1. On addition of acid or alkali, a precipitate forms when the hydrogen-ion concentration becomes too high or too low. This precipitate forms on the alkaline side at about pH 8 and becomes greater as the hydrogen-ion concentration is further reduced to pH 12, the largest amount of precipitate occurring at pH 12. Since conditions at pH 8 to pH 12 are unfavorable for adsorption of virus, removal of this precipitate by filtration or centrifugation could be used as a clarification process without great loss of virus. In the case of the precipitate that forms on the acid side, when the hydrogen-ion concentration is increased to about pH 2, conditions favor adsorption of virus, hence removal of precipitate would also tend to remove virus. Therefore, the precipitates were allowed to remain and the virus preparations were well stirred in order to distribute the precipitates evenly before removal of samples for test purposes.

Immediately after adjustment to the various hydrogen-ion concentrations, the main portion of each of the preparations and an untreated control were placed in a room held at −14° C. After standing for 2 days, the 12 preparations and the control were removed, allowed to thaw, and the hydrogen-ion concentration of each was determined. The results, listed

TABLE 1.—*Record of amount of acid or alkali required to adjust preparations, of hydrogen-ion concentrations at the end of various time periods, and of amount of acid or alkali required to readjust preparations*

| Amount of diluted untreated juice used | Amount of acid or alkali used | pH of adjusted preparation | pH after 2 days. Before readjustment | pH after 9 days. Before readjustment | pH after 36 days. Before readjustment | pH after 4 months. Before readjustment | pH after 12 months. Before readjustment | Amount of 2N acid or alkali used to adjust 50 cc. of each preparation to pH $6.0 \pm 0.2$ |
|---|---|---|---|---|---|---|---|---|
| 400 cc. | 5 cc. conc. HCl + 15 cc. 2N HCl | 1.00 | 1.06 | 1.05 | 1.09 | 0.93 | ..... | 4.02 cc. |
| 550 cc. | 15 cc. 2N HCl | 2.01 | 2.05 | 2.07 | 2.02 | 2.06 | ..... | 1.47 cc. |
| 300 cc. | 4 cc. 2N HCl | 3.05 | 3.11 | 3.02 | 2.94 | 3.00 | 3.03 | 0.60 cc. |
| 300 cc. | 2.2 cc. 2N HCl | 4.00 | 4.12 | 3.98 | 3.89 | 3.98 | 4.00 | 0.40 cc. |
| 300 cc. | 0.44 cc. 2N HCl | 5.04 | 5.12 | 5.00 | 4.88 | 5.06 | 5.04 | 0.08 cc. |
| 300 cc. | 0.12 cc. 2N NaOH | 6.03 | 6.09 | 5.94 | 5.90 | 6.22 | 6.04 | ..... |
| 300 cc. | 0.54 cc. 2N NaOH | 7.05 | 7.20 | 6.89 | 6.89 | 7.11 | 7.05 | 0.05 cc. |
| 550 cc. | 1.5 cc. 2N NaOH | 8.00 | 8.00 | 7.75 | 7.74 | 7.97 | 7.85 | 0.10 cc. |
| 550 cc. | 2.08 cc. 2N NaOH | 9.01 | 8.88 | 8.60 | 8.67 | 8.88 | 8.50 | 0.20 cc. |
| 300 cc. | 2.0 cc. 2N NaOH | 9.97 | 9.70 | 9.66 | 9.37 | 9.80 | 9.75 | 0.59 cc. |
| 300 cc. | 3.5 cc. 2N NaOH | 11.05 | 10.98 | 10.77 | 10.21 | 11.15 | 10.81 | 0.69 cc. |
| 300 cc. | 15.0 cc. 2N NaOH | 12.00 | 12.07 | 12.03 | 11.46 | 11.62 | 11.35 | 2.06 cc. |

in the 4th column of table 1, indicate that some of the preparations were more than 0.05 pH from the desired hydrogen-ion concentrations. These preparations were, therefore, readjusted to within 0.05 pH of the desired values. Additional 50 c.. portions of each were removed, adjusted to pH 6.0 ± 0.2, and tested on *Phaseolus vulgaris* and *Nicotiana glutinosa* against the control. The main portion of each preparation was again placed in a room held at −14° C. At the end of 9 days, 36 days, 4 and 12 months, the above-described procedure was repeated. The hydrogen-ion concentrations of the various preparations before readjustment and at the end of the various time periods are given in columns 5 to 8, inclusive, of table 1. It may be seen that the preparations at pH 1 to pH 9 remained fairly close to the desired values, but that the preparations at pH 10, pH 11, and pH 12 became changed by as much as 0.79 pH. This marked change occurred during the longer time periods when all or most of the virus had already been inactivated and did not, therefore, introduce serious errors.

The result of the tests for virus infectivity are given in table 2. The actual number of lesions per half-leaf obtained with each preparation at the end of each period of time is given for every preparation. An averaged number which is the quotient of the number of lesions obtained per half-leaf on inoculation with the various preparations, divided by the number of lesions obtained on the other halves of the same leaves on inoculation with the control or untreated virus preparation, is also given. When this averaged number approximates 100, it indicates that the preparation produced about as many lesions as did the control preparation, and that little or no inactivation of virus occurred. As this number decreases, it indicates that a decreasing amount of virus is present. When no lesions are produced on inoculation of *Nicotiana glutinosa* or *Phaseolus vulgaris*, the quotient is zero and inactivation is complete. Since the control was the same in all instances, each number is related to all other numbers. This makes possible a comparison of the amount of virus, as evidenced by the number of lesions produced on inoculation, remaining in each of the preparations at the end of the different time periods. It should be emphasized, however, that as others (2, 4, 5, 8, 9, 13, 14) have indicated, the relation of number of lesions to virus concentration does not approach a direct proportionality, except possibly at high dilutions. An idea of the actual relative amount of virus present can be obtained only by comparison of the number of lesions produced by any given sample with the numbers of lesions produced by dilutions of the control virus sample, the relative concentrations of which are known. It is also necessary that the virus preparation be sufficiently diluted so that on further dilution fewer lesions are produced on inoculation. The original virus preparations used in all experiments described in this paper were appropriately diluted. If, therefore, under

TABLE 2.—*Tests for virus infectivity of diluted untreated infectious juice at different hydrogen-ion concentrations immediately after ad-*

TABLE 2.—*Tests for virus infectivity of diluted untreated infectious juice at different hydrogen-ion concentrations immediately after adjustment and at pH 6, after standing at different hydrogen-ion concentrations at − 14° C. for periods of time up to 1 year*

| Preparations maintained at pH | Test plant | Preparations tested at different pH's immediately after adjustment | Preparations tested at pH 6 after being held 1 hour at different pH's | | Preparations tested at pH 6 after being held 2 days at different pH's | | Preparations tested at pH 6 after being held 9 days at different pH's | | Preparations tested at pH 6 after being held 36 days at different pH's | | Preparations tested at pH 6 after being held 4 months at different pH's | | Preparations tested at pH 6 after being held 12 months at different pH's | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Actual | Averaged[a] | Actual[b] | Averaged | Actual | Averaged | Actual | Averaged | Actual | Averaged | Actual | Averaged | Actual |
| 1 | N. glutinosa | | 37.1 | 30.8 | 2.2 | 2.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | P. vulgaris | 0.12 | 15.6 | 14.3 | 0.1 | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 2 | N. glutinosa | | 82.3 | 58.3 | 37.6 | 35.5 | 2.8 | 2.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | P. vulgaris | 4.7 | 74.0 | 69.8 | 27.1 | 29.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 3 | N. glutinosa | | 106.0 | 87.5 | 97.2 | 78.5 | 80.5 | 79.4 | 95.0 | 98.4 | 74.0 | 24.8 | 79.5 | 78.6 |
| | P. vulgaris | 6.3 | 69.4 | 58.5 | 87.5 | 94.7 | 165.0 | 89.0 | 154.0 | 71.7 | 69.6 | 19.9 | 88.5 | 9.2 |
| 4 | N. glutinosa | | 114.1 | 55.8 | 85.8 | 67.4 | 102.8 | 125.0 | 124.6 | 98.0 | 64.5 | 39.7 | 75.0 | 70.7 |
| | P. vulgaris | 23.2 | 88.0 | 70.1 | 91.5 | 147.0 | 145.0 | 71.7 | 178.0 | 132.0 | 72.1 | 34.2 | 98.0 | 8.3 |
| 5 | N. glutinosa | | 98.1 | 76.8 | 140.5 | 112.0 | 104.0 | 98.8 | 72.1 | 86.9 | 94.5 | 56.0 | 96.5 | 86.4 |
| | P. vulgaris | 55.6 | 88.3 | 53.2 | 90.0 | 116.2 | 146.0 | 91.5 | 125.7 | 103.7 | 69.6 | 37.6 | 82.5 | 13.4 |
| 6 | N. glutinosa | | 100.8 | 43.0 | 132.5 | 79.4 | 116.1 | 94.6 | 109.8 | 79.5 | 89.0 | 63.0 | 90.0 | 96.6 |
| | P. vulgaris | 74.0 | 91.2 | 82.1 | 80.0 | 121.1 | 127.7 | 87.0 | 166.0 | 85.5 | 74.9 | 27.6 | 73.4 | 7.6 |
| 7 | N. glutinosa | | 92.3 | 79.2 | 105.7 | 49.7 | 99.8 | 106.5 | 98.6 | 86.9 | 128.0 | 94.0 | 86.0 | 95.7 |
| | P. vulgaris | 71.5 | 99.8 | 55.5 | 79.5 | 105.0 | 110.0 | 71.0 | 162.2 | 107.0 | 95.5 | 34.5 | 86.0 | 9.7 |
| 8 | N. glutinosa | | 112.1 | 83.2 | 112.0 | 64.6 | 80.0 | 55.6 | 112.2 | 74.6 | 91.0 | 66.5 | 94.5 | 73.4 |
| | P. vulgaris | 98.9 | 79.0 | 62.5 | 69.5 | 101.0 | 143.7 | 96.5 | 156.0 | 68.5 | 71.5 | 13.7 | 43.0 | 1.8 |
| 9 | N. glutinosa | | 94.4 | 70.9 | 88.9 | 74.8 | 91.5 | 60.0 | 60.3 | 63.7 | 48.4 | 31.3 | 37.9 | 19.9 |
| | P. vulgaris | 92.5 | 72.9 | 74.1 | 65.8 | 71.6 | 98.5 | 61.3 | 71.7 | 39.1 | 40.2 | 7.5 | 22.1 | 0.8 |
| 10 | N. glutinosa | | 79.2 | 70.0 | 49.2 | 43.0 | 26.4 | 18.0 | 9.7 | 11.0 | 3.3 | 2.8 | 1.2 | 1.9 |
| | P. vulgaris | 59.8 | 68.5 | 36.4 | 22.3 | 29.6 | 11.0 | 7.2 | 0.8 | 0.5 | 0.0 | 0.0 | 0.0 | 0.0 |
| 11 | N. glutinosa | | 0.4 | 0.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | P. vulgaris | 11.4 | 0.2 | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 12 | N. glutinosa | | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | P. vulgaris | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

[a] Numbers represent the quotient of the average number of lesions per half-leaf obtained on 5 or more half-leaves of *Nicotiana glutinosa* or 12 or more half-leaves of *Phaseolus vulgaris* on inoculation with the designated preparation, divided by the average number of lesions obtained on the other halves of the same leaves on inoculation with the control preparation.

[b] Numbers represent the average of the actual number of lesions per half-leaf obtained on 5 or more half-leaves of *N. glutinosa* or 12 or more half-leaves of *P. vulgaris* on inoculation with the designated preparation.

the same conditions the sample produces about the same number of lesions as the control diluted 1 to 10, it may be assumed that about 90 per cent of the virus has been inactivated. If the sample produces about the same number of lesions as the control diluted 1 to 100, then about 99 per cent of the virus has been inactivated. Tests of dilutions of the control preparations by the half-leaf method are given in table 5 in order to permit estimation of the actual amount of virus present in the various preparations. An idea of the rate of inactivation of virus at different hydrogen-ion concentrations may thus be obtained.

The results given in table 2 show that tobacco-mosaic virus is inactivated immediately at pH 12, very rapidly at pH 11 and at pH 1, and fairly rapidly at pH 10 and pH 2. The results also indicate that the virus is quite stable at pH 3 to pH 8 inclusive, and fairly stable at pH 9, for periods as long as 1 year. The rate of inactivation of tobacco-mosaic virus in diluted untreated juice prepared from frozen plants is, therefore, extremely slow at hydrogen-ion concentrations between pH 3 and pH 8, and very rapid at hydrogen-ion concentrations more acid than pH 3 and more alkaline than pH 9. Virus preparations completely inactivated at pH 1, pH 2, pH 11, and pH 12, and then adjusted to pH 6 were tested for virus infectivity after standing for periods of time as long as 1 year, and in no case was virus demonstrated. This is an indication that virus inactivated at unfavorable hydrogen-ion concentrations, such as pH 1, pH 2, pH 11, and pH 12, does not become reactivated on long standing at pH 6, a hydrogen-ion concentration that may be considered favorable to the virus.

### Rates of Inactivation of Virus in Juice from Macerated Fresh Leaves in Critical Regions near pH 2 and pH 10

In the experiment just described, no tests were included for periods between 1 hour and 48 hours and only 6 preparations were at hydrogen-ion concentrations more acid than pH 3.5 and more alkaline than pH 9.5, the two regions in which the rate of inactivation becomes very rapid. It seemed desirable to make tests at shorter time intervals and at a greater number of different hydrogen-ion concentrations in the regions near pH 2 and pH 10. Since some investigators work at room temperature with virus extracts prepared from freshly macerated tissues, it seemed desirable to make tests on such extracts held at room temperature. An experiment was made, therefore, for the purpose of determining the rates of inactivation at room temperature (20° C.), in the regions around pH 2 and pH 10, of virus in juice prepared from fresh tissue. Leaves from plants of *Nicotiana tabacum* variety Turkish, diseased with tobacco mosaic, were removed, put through a meat grinder, and the juice pressed out.

This juice, which was at pH 5.7, was centrifuged, diluted with 2 volumes of distilled water, and saturated with toluol. After standing overnight at room temperature, the hydrogen-ion concentration of the preparation was pH 7.1. Thirteen 300 cc. portions of this preparation were held at room temperature (20° C.) for 192 hours, at hydrogen-ion concentrations of from pH 0.5 to pH 3.5 and from pH 9.5 to pH 12 in gradations of 0.5 pH. The adjustment of the preparations to the desired hydrogen-ion concentrations and the readjustment of portions of each to, in this experiment, pH 6.0 $\pm$ 0.4 was done in exactly the same manner as that described for the first experiment. The amounts of acid and alkali required were comparable to the amounts used in the first experiment. The precipitates which formed in these preparations were well mixed before removal of samples for test purposes.

Tests for virus infectivity were made by the half-leaf method on each preparation immediately after adjustment to the different hydrogen-ion concentrations, at these hydrogen-ion concentrations, and also after being readjusted to pH 6.0 $\pm$ 0.4. Tests were also made at pH 6.0 $\pm$ 0.4 on preparations that had stood at room temperature at the various hydrogen-ion concentrations for time periods of 1, 5, 20, 48, 96, and 192 hours. In this experiment the different preparations were not readjusted to the initial hydrogen-ion concentrations at the end of the various time periods, since the rate of hydrolysis at pH 9.5 to pH 12 at room temperature was found to be so great as to cause the preparations to require readjustment every few hours in order to maintain fairly constant hydrogen-ion concentrations. It may be seen from table 3 that preparations initially at pH 9.5 to pH 12 became changed by as much as 2.1 pH during the course of 8 days. The results obtained with preparations at pH 9.5 to pH 11 are, therefore, indicative of inactivation in preparations initially adjusted to the given hydrogen-ion concentrations and then allowed to stand without further treatment, and not of preparations held at constant hydrogen-ion concentrations. The results obtained with preparations at all other hydrogen-ion concentrations are, however, indicative of inactivation in preparations held at constant hydrogen-ion concentrations, since, at pH 11.5 and pH 12.0, inactivation of virus was completed before the hydrogen-ion concentration changed appreciably, and at pH 0.5 to pH 3.5 there was but little change in the hydrogen-ion concentration of the preparations during the course of 8 days. The results of the tests for virus infectivity (Table 3) show that the virus is remarkably stable between pH 1.5 and pH 10.0 for periods as long as 20 hours and even somewhat stable at pH 1.0, pH 10.5, and pH 11.0 for the same length of time. It is completely inactivated at pH 12 and almost completely inactivated at pH 0.5 immediately after adjustment to these hydrogen-ion concentrations. At pH

TABLE 3.—*Tests for virus infectivity at different hydrogen-ion concentrations immediately after adjustment and at pH 6, after standing at different hydrogen-ion concentrations at 20° C. for periods of time up to 192 hours. Untreated infectious juice prepared from fresh leaves*

| pH of preparations at start | Test plant | Preparations tested at different pH's immediately after adjustment | | Preparations tested after an immediate readjustment to pH 6 | | Preparations tested at pH 6 after being held 1 hour at different pH's | | Preparations tested at pH 6 after being held 5 hours at different pH's | | Preparations tested at pH 6 after being held 20 hours at different pH's | | Preparations tested at pH 6 after being held 48 hours at different pH's | | Preparations tested at pH 6 after being held 96 hours at different pH's | | Preparations tested at pH 6 after being held 192 hours at different pH's | | pH of preparations at end of 8 days |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Averaged[a] | Actual[b] | Averaged | Actual | Averaged | Actual | Averaged | Actual | Averaged | Actual | Averaged | Actual | Averaged | Actual | Averaged | Actual | |
| 0.5 | N. glutinosa | 0.0 | 0.0 | 0.1 | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.6 |
| | P. vulgaris | 0.0 | 0.0 | | | | | | | | | | | | | | | |
| 1.0 | N. glutinosa | 4.6 | 8.8 | 53.4 | 104.6 | 59.5 | 139.8 | 61.0 | 102.6 | 56.5 | 85.8 | 1.3 | 1.7 | 0.0 | 0.0 | 0.0 | 0.0 | 1.1 |
| | P. vulgaris | 0.0 | 0.0 | | | | | | | | | 1.0 | 0.4 | | | | | |
| 1.5 | N. glutinosa | 23.1 | 35.8 | 85.6 | 138.2 | 96.0 | 232.8 | 85.0 | 204.2 | 82.5 | 139.0 | 50.5 | 86.0 | 50.4 | 107.2 | 56.0 | 121.0 | 1.5 |
| | P. vulgaris | 1.1 | 0.3 | | | | | | | | | 79.0 | 50.5 | | | | | |
| 2.0 | N. glutinosa | 74.8 | 137.0 | 73.5 | 149.4 | 88.4 | 177.4 | 98.5 | 241.2 | 69.6 | 131.6 | 90.8 | 161.8 | 55.4 | 95.6 | 58.0 | 122.0 | 2.0 |
| | P. vulgaris | 6.8 | 1.7 | | | | | | | | | 140.0 | 75.5 | | | | | |
| 2.5 | N. glutinosa | 87.0 | 194.4 | 70.4 | 184.4 | 83.2 | 157.6 | 83.0 | 208.0 | 77.2 | 106.8 | 115.0 | 179.8 | 84.5 | 180.0 | 78.0 | 127.0 | 2.5 |
| | P. vulgaris | 11.5 | 3.0 | | | | | | | | | 184.0 | 52.4 | | | | | |
| 3.0 | N. glutinosa | 77.7 | 169.2 | 65.4 | 126.4 | 77.0 | 191.8 | 79.4 | 267.0 | 90.5 | 137.8 | 109.1 | 206.6 | 90.0 | 159.2 | 81.3 | 179.6 | 3.0 |
| | P. vulgaris | 10.4 | 4.2 | | | | | | | | | 159.0 | 75.9 | | | | | |
| 3.5 | N. glutinosa | 101.0 | 160.0 | 83.8 | 95.0 | 90.8 | 151.8 | 95.5 | 158.8 | 99.0 | 109.6 | 100.0 | 136.6 | 82.0 | 148.0 | 96.5 | 113.4 | 3.5 |
| | P. vulgaris | 10.6 | 0.8 | | | | | | | | | 435.0 | 85.0 | | | | | |
| 9.5 | N. glutinosa | 86.5 | 144.0 | 97.2 | 166.6 | 112.7 | 122.6 | 102.5 | 188.4 | 95.0 | 139.0 | 92.5 | 183.8 | 63.8 | 124.6 | 74.2 | 118.4 | 8.5 |
| | P. vulgaris | 199.5 | 59.8 | | | | | | | | | 66.5 | 17.7 | | | | | |
| 10.0 | N. glutinosa | 92.5 | 186.8 | 94.8 | 179.8 | 77.5 | 136.4 | 86.0 | 201.2 | 91.5 | 142.6 | 82.2 | 166.0 | 83.0 | 166.4 | 71.3 | 97.4 | 8.6 |
| | P. vulgaris | 485.0 | 121.0 | | | | | | | | | 10.0 | 0.6 | | | | | |
| 10.5 | N. glutinosa | 84.6 | 209.2 | 81.4 | 92.4 | 67.7 | 138.4 | 74.2 | 159.8 | 36.4 | 67.8 | 34.5 | 43.4 | 63.6 | 110.8 | 39.2 | 26.8 | 8.9 |
| | P. vulgaris | 323.0 | 28.4 | | | | | | | | | 10.3 | 3.4 | | | | | |
| 11.0 | N. glutinosa | 56.8 | 111.6 | 80.5 | 189.4 | 78.0 | 117.2 | 76.7 | 138.6 | 49.6 | 97.2 | 27.6 | 52.8 | 50.6 | 110.0 | 46.5 | 60.8 | 9.2 |
| | P. vulgaris | 55.1 | 8.4 | | | | | | | | | 2.4 | 0.7 | | | | | |
| 11.5 | N. glutinosa | 1.7 | 3.4 | 35.8 | 84.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 9.4 |
| | P. vulgaris | 0.0 | 0.0 | | | | | | | | | | | | | | | |
| 12.0 | N. glutinosa | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 11.1 |
| | P. vulgaris | 0.0 | 0.0 | | | | | | | | | | | | | | | |

[a] Numbers represent the quotient of the average number of lesions per half-leaf obtained on 5 or more half-leaves of *Nicotiana glutinosa* or 12 or more half-leaves of *Phaseolus vulgaris* on inoculation with the designated preparation, divided by the average number of lesions obtained on the other halves of the same leaves on inoculation with the control preparation.

[b] Numbers represent the average of the actual number of lesions per half-leaf obtained on 5 or more half-leaves of *N. glutinosa* or 12 or more half-leaves of *P. vulgaris* on inoculation with the designated preparation.

1.0, standing for between 48 and 96 hours was required for complete inactivation, while at pH 11.5 less than 1 hour was required for complete inactivation. Preparations held at pH 1.5 for as long as 192 hours still gave about 50 per cent as many lesions as the untreated control. It is evident that the virus may be subjected to a very wide range of hydrogen-ion concentrations for fairly long periods of time during chemical manipulation without much loss of activity.

### Rates of Inactivation of Virus in Purified Preparations

In order to determine whether the large amount of extraneous matter present in untreated juice has an effect on the rate of inactivation of virus, a third experiment was made using a purified preparation of virus in place of diluted untreated infectious juice. A 350 cc. portion of a virus preparation purified by a modification of the lead acetate process described by Vinson and Petre (16) was diluted to 3.5 liters with 0.1 M phosphate at pH 6.8, and 35 g. of charcoal (Suchar 00) was added. After stirring for 5 minutes, the mixture was filtered through hardened filter paper 4 times. Then 250 cc. portions were taken to different hydrogen-ion concentrations, readjusted to about pH 6.8 after standing for different periods of time at −14° C., and tested on *Phaseolus vulgaris* and *Nicotiana glutinosa* in exactly the same manner as described in the first experiment in which diluted untreated infectious juice was used. Due to the absence of the large amount of extraneous materials capable of acid or alkaline hydrolysis, the purified preparations remained very close to the desired hydrogen-ion concentrations and only slight precipitates were formed in the preparations at high or low hydrogen-ion concentrations. The preparations were usually within 0.05 pH unit and never over 0.2 pH unit from the desired hydrogen-ion concentration at the end of the various time periods. The amounts of acid and alkali used to adjust and readjust the hydrogen-ion concentrations are not recorded, since they are only the amounts necessary to take care of the 0.1 M phosphate buffer. The virus and other materials present have no noticeable buffering capacity at the dilution used.

The results of the tests (Table 4) indicate that, in general, the rate of inactivation of virus in purified preparations is about the same as that of virus in diluted untreated juice except at low hydrogen-ion concentrations, such as pH 11 and pH 12, where the virus in purified preparations appears to be more stable. It may be seen from table 4 that the preparations at pH 9 and pH 10 still gave more than 50 per cent as many lesions as the control even at the end of 1 year. The purified preparation withstood momentary adjustment to pH 11 without appreciable loss of activity and even to pH 12 without complete loss of activity. Using the data given in table 5, it is possible to estimate the amount of virus remaining in the

lation. Numbers represent the average of the actual number of lesions per half-leaf obtained on 5 or more half-leaves of *N. glutinosa* or 12 or more half-leaves of *P. vulgaris* on inoculation with the designated preparation.

TABLE 4.—*Tests for virus infectivity of a purified virus preparation at different hydrogen-ion concentrations immediately after adjustment and at pH 6.8, after standing at different hydrogen-ion concentrations at −14° C. for periods of time up to 1 year*

| Preparations maintained at pH | Test plant | Preparations tested at different pH's immediately after adjustment | | Preparations tested after an immediate readjustment to pH 6.8 | | Preparations tested at pH 6.8 after being held 1 hour at different pH's | | Preparations tested at pH 6.8 after being held 2 days at different pH's | | Preparations tested at pH 6.8 after being held 9 days at different pH's | | Preparations tested at pH 6.8 after being held 32 days at different pH's | | Preparations tested at pH 6.8 after being held 5 months at different pH's | | Preparations tested at pH 6.8 after being held 12 months at different pH's | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Averaged[a] | Actual[b] | Averaged | Actual | Averaged | Actual | Averaged | Actual | Averaged | Actual | Averaged | Actual | Averaged | Actual | Averaged | Actual |
| 1 | N. glutinosa | 7.5 | 3.6 | 62.7 | 71.6 | 56.8 | 23.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | P. vulgaris | 2.3 | 1.2 | 91.5 | 116.0 | 61.2 | 41.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 2 | N. glutinosa | 35.6 | 11.7 | 84.7 | 59.6 | 114.7 | 58.8 | 65.5 | 81.4 | 7.9 | 5.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | P. vulgaris | 7.8 | 8.8 | 85.9 | 157.5 | 102.1 | 104.0 | 126.2 | 158.0 | 8.3 | 10.8 | 0.1 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 |
| 3 | N. glutinosa | 84.6 | 51.8 | 103.1 | 76.4 | 85.5 | 80.1 | 164.9 | 95.7 | 99.0 | 57.5 | 63.8 | 67.6 | 6.0 | 5.5 | 2.0 | 2.3 |
| | P. vulgaris | 27.4 | 19.4 | 98.2 | 133.2 | 100.8 | 139.0 | 143.0 | 149.1 | 95.0 | 93.0 | 74.0 | 148.0 | 2.9 | 2.3 | 1.6 | 3.1 |
| 4 | N. glutinosa | 85.0 | 51.4 | 98.2 | 82.1 | 144.0 | 59.0 | 133.0 | 87.7 | 94.0 | 58.5 | 92.3 | 107.0 | 128.0 | 90.4 | 89.1 | 88.4 |
| | P. vulgaris | 80.1 | 85.8 | 95.7 | 122.8 | 93.1 | 89.2 | 150.0 | 214.0 | 78.9 | 104.9 | 94.5 | 148.2 | 95.8 | 102.0 | 92.0 | 122.9 |
| 5 | N. glutinosa | 83.8 | 36.4 | 104.2 | 101.2 | 100.0 | 78.9 | 166.0 | 75.6 | 85.5 | 50.6 | 86.7 | 144.0 | 100.0 | 53.5 | 80.9 | 72.4 |
| | P. vulgaris | 93.0 | 100.0 | 110.3 | 142.3 | 101.8 | 95.9 | 136.0 | 179.0 | 80.0 | 106.5 | 80.4 | 142.0 | 109.0 | 141.5 | 86.5 | 105.8 |
| 6 | N. glutinosa | 118.0 | 44.1 | 101.3 | 89.8 | 108.9 | 55.0 | 109.2 | 86.4 | 102.0 | 114.7 | 67.1 | 147.8 | 103.0 | 68.8 | 91.6 | 81.2 |
| | P. vulgaris | 135.0 | 171.5 | 96.2 | 128.3 | 97.5 | 139.0 | 138.0 | 132.8 | 94.1 | 97.9 | 96.5 | 183.3 | 100.0 | 119.6 | 84.5 | 106.6 |
| 7 | N. glutinosa | 129.2 | 76.4 | 108.4 | 82.8 | 119.0 | 93.2 | 95.0 | 60.8 | 102.5 | 60.4 | 64.8 | 143.0 | 90.0 | 71.0 | 97.6 | 92.3 |
| | P. vulgaris | 140.0 | 219.8 | 89.2 | 132.1 | 139.5 | 137.2 | 137.0 | 133.0 | 81.0 | 124.0 | 73.0 | 156.0 | 115.0 | 179.5 | 83.5 | 106.2 |
| 8 | N. glutinosa | 128.4 | 42.4 | 96.3 | 84.1 | 116.0 | 82.0 | 128.4 | 72.5 | 95.8 | 74.4 | 72.6 | 131.0 | 74.3 | 51.3 | 83.1 | 76.7 |
| | P. vulgaris | 109.8 | 141.8 | 104.1 | 163.8 | 121.5 | 202.0 | 140.0 | 140.0 | 88.8 | 114.5 | 63.0 | 143.0 | 76.8 | 111.0 | 89.0 | 95.2 |
| 9 | N. glutinosa | 160.0 | 40.8 | 115.0 | 47.4 | 113.0 | 119.0 | 127.8 | 59.0 | 87.6 | 67.1 | 54.7 | 132.5 | 62.4 | 45.6 | 71.1 | 62.8 |
| | P. vulgaris | 132.4 | 216.0 | 76.5 | 182.2 | 93.0 | 77.0 | 128.5 | 108.0 | 83.4 | 111.5 | 63.3 | 187.8 | 84.6 | 82.0 | 96.5 | 87.5 |
| 10 | N. glutinosa | 80.0 | 60.3 | 76.3 | 64.1 | 92.5 | 97.2 | 70.5 | 29.2 | 67.6 | 65.0 | 61.2 | 76.5 | 53.2 | 25.6 | 46.3 | 31.8 |
| | P. vulgaris | 111.3 | 196.5 | 70.3 | 144.2 | 80.0 | 95.0 | 133.1 | 119.8 | 60.7 | 92.1 | 65.4 | 106.0 | 86.5 | 80.5 | 50.5 | 56.9 |
| 11 | N. glutinosa | 82.8 | 58.2 | 103.0 | 79.8 | 50.8 | 19.6 | 4.4 | 2.2 | 0.4 | 0.3 | 0.1 | 0.2 | 0.0 | 0.0 | 0.0 | 0.0 |
| | P. vulgaris | 112.0 | 151.3 | 90.1 | 124.7 | 50.1 | 56.6 | 5.0 | 5.3 | 0.4 | 0.5 | 0.1 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 |
| 12 | N. glutinosa | 0.0 | 0.0 | 1.1 | 1.3 | 1.3 | 0.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | P. vulgaris | 0.0 | 0.0 | 0.2 | 0.2 | 1.1 | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

[a] Numbers represent the quotient of the average number of lesions per half-leaf obtained on 5 or more half-leaves of *Nicotiana glutinosa* or 12 or more half-leaves of *Phaseolus vulgaris* on inoculation with the designated preparation, divided by the average number of lesions obtained on the other halves of the same leaves on inoculation with the control preparation.

[b] Numbers represent the average of the actual number of lesions per half-leaf obtained on 5 or more half-leaves of *N. glutinosa* or 12 or more half-leaves of *P. vulgaris* on inoculation with the designated preparation.

various samples. The rate of inactivation of virus in the purified samples is so slow as to be negligible at pH 4 to pH 8, very slow at pH 9 and pH 10, fairly rapid at pH 2, pH 3 and pH 11, and very rapid at pH 1 and pH 12. The virus preparations which were completely inactivated at pH 1, pH 2, pH 11, and pH 12 and then adjusted to pH 6.8 were again tested for virus infectivity after standing for periods of time as long as 1 year, and in no instance was it possible to demonstrate the presence of active virus.

### Inactivation of Tobacco-ring-spot and Cucumber-mosaic Viruses at Different Hydrogen-ion Concentrations[2]

Since tobacco-mosaic virus is more stable, in many respects (10), than other viruses, such as tobacco-ring-spot and cucumber-mosaic, it seemed desirable to determine, for purposes of comparison, whether these two viruses are more or less stable at different hydrogen-ion concentrations than tobacco-mosaic virus. Experiments were made, therefore, to determine the effect of standing at different hydrogen-ion concentrations for 1 hour at room temperature on the inactivation of tobacco-ring-spot (Wingard strain) and cucumber-mosaic virus.

Infectious juice from plants of *Nicotiana tabacum* variety Turkish infected with ring-spot virus was diluted with 4 parts of distilled water and 50 cc. portions were taken to pH 2 to pH 12 in 1 pH gradations by the addition of 2 N NaOH or HCl with vigorous stirring. The prepara-

TABLE 5.—*Tests for virus infectivity of dilutions of control preparations*

| Dilution | Tested on *Phaseolus vulgaris* | | Tested on *Nicotiana glutinosa* | |
|---|---|---|---|---|
| | Diluted[a] untreated juice | Purified[b] virus preparation | Diluted[a] untreated juice | Purified[b] virus preparation |
| 1 | 67.5[c] | 161.0[d] | 107.0[c] | 179.0[d] |
| $1 \times 10^{-1}$ | 20.1 | 125.3 | 17.7 | 43.8 |
| $1 \times 10^{-2}$ | 3.5 | 74.8 | 3.4 | 12.9 |
| $1 \times 10^{-3}$ | 0.2 | 24.0 | 1.0 | 7.5 |
| $1 \times 10^{-4}$ | 0.4 | 8.1 | 0.0 | 1.4 |

[a] Dilutions were made with distilled water.

[b] Dilutions were made with 0.1 M phosphate at pH 6.8.

[c] Numbers represent the average number of lesions per half-leaf obtained on the left halves of leaves on inoculation with the designated preparation and dilution.

[d] Numbers represent the average number of lesions per half-leaf obtained on the right halves of the same leaves on inoculation with the designated preparation and dilution.

[2] These experiments were made in collaboration with Dr. W. C. Price, who carried out the tests for virus infectivity.

tions at the 10 different hydrogen-ion concentrations were tested immediately after adjustment for virus infectivity on cowpea, *Vigna sinensis* (L.) Endl. var. Black Eye. After standing for 1 hour at room temperature (20° C.), the preparations were readjusted to the hydrogen-ion concentration of the untreated preparation (pH 6.02) and again tested for virus infectivity. The results of the tests (Table 6) show that the virus is completely inactivated after standing for 1 hour at pH 2, pH 10

TABLE 6.—*Tests for virus infectivity on cowpea of a preparation of tobacco-ring-spot virus at different hydrogen-ion concentrations immediately after adjustment and at pH 6 after standing for 1 hour*

| Preparations held at pH → | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
|---|---|---|---|---|---|---|---|---|---|---|
| Preparations tested at different pH's immediately after adjustment | 0.00[a] | 0.00 | 0.05 | 0.35 | 9.40 | 14.10 | 19.70 | 5.39 | 0.00 | 0.00 |
| Preparations tested at pH 6 after being held 1 hour at different pH's | 0.00 | 0.10 | 1.05 | 2.40 | 11.30 | 5.29 | 6.50 | 0.32 | 0.00 | 0.00 |

[a] Numbers represent the average number of lesions per leaf obtained on 40 leaves of cowpea, *Vigna sinensis* var. Black Eye, on inoculation with the designated preparation.

and pH 11. It is also almost completely inactivated after standing for an hour at pH 3 or pH 9. The results indicate that the virus tends to inactivate as the hydrogen-ion concentration is increased or decreased from pH 6. The increased number of lesions produced on immediate inocula-

TABLE 7.—*Tests for virus infectivity on cowpea of a preparation of cucumber-mosaic virus at different hydrogen-ion concentrations immediately after adjustment and at pH 5.7 after standing for 1 hour*

| Preparations held at pH → | Dilution[a] | 3 | 5 | 5.7 | 7 | 9 |
|---|---|---|---|---|---|---|
| Preparations tested at different pH's immediately after adjustment | Undiluted | 0.17[b] | 3.19 | 1.87 | 2.85 | 1.85 |
| | $1 \times 10^{-1}$ | 0.00 | 0.43 | 0.30 | 0.40 | 1.12 |
| | $1 \times 10^{-2}$ | 0.00 | 0.00 | 0.05 | 0.00 | 0.06 |
| Preparations tested at pH 5.7 after being held 1 hour at different pH's | Undiluted | 0.19 | 1.58 | 2.94 | 1.67 | 0.63 |
| | $1 \times 10^{-1}$ | 0.00 | 0.17 | 0.31 | 0.28 | 0.07 |
| | $1 \times 10^{-2}$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

[a] Dilutions were made with distilled water.

[b] Numbers represent the average number of lesions per leaf obtained on 40 leaves of cowpea, *Vigna sinensis* var. Black Eye, on inoculation with the designated preparation.

tion at pH 7 and pH 8 and the decreased number at pH 5, pH 4 and pH 3 is a phenomenon similar to that encountered with tobacco-mosaic virus on *Phaseolus vulgaris*.

Portions of 30 cc. each of infectious juice at pH 5.7 from plants of *Nicotiana tabacum* variety Turkish infected with ordinary cucumber-mosaic were taken to pH 3, pH 5, pH 7 and pH 9 and immediately tested with the untreated control for virus infectivity on cowpea, *Vigna sinensis* variety Black Eye. After standing for 1 hour at 20° C., the preparations were readjusted to pH $5.7 \pm 0.1$ and again tested for virus infectivity. The results, which are given in table 7, are in general similar to the results obtained with ring-spot virus. The cucumber-mosaic virus is inactivated as the hydrogen-ion concentration is increased or decreased from pH 5.7. Ring-spot and cucumber-mosaic viruses are, therefore, much less stable than tobacco-mosaic virus as the hydrogen-ion concentration is increased or decreased from about pH 6.

## DISCUSSION

The experiments described in this paper show that the rate of inactivation of tobacco-mosaic virus in diluted untreated infectious juice at room temperature or $-14°$ C. is so slow as to be negligible between pH 3 and pH 8, fairly rapid between pH 1.5 and pH 2.5 and between pH 9 and pH 10, and very rapid between pH 0.5 and pH 1.5 and between pH 11 and pH 12.

It is sometimes desirable, in making a comparative study of the properties of a virus, to be able to remove different amounts of virus from a given preparation in order to study the properties of the remaining virus. This may be accomplished easily by adjusting virus preparations to hydrogen-ion concentrations at which the rate of inactivation is fairly rapid and readjusting portions of the preparation to a favorable hydrogen-ion concentration after different periods of time. Any desired percentage of the original amount of virus may be obtained by adjusting the hydrogen-ion concentration and the time of exposure. It should be noted that the amount of acid or alkali necessary to adjust a virus sample to a given hydrogen-ion concentration varies with the buffering capacity of the virus preparation. This may vary considerably, depending largely upon the method used to prepare the virus sample. In general, preparations containing large amounts of solids have a greater buffering capacity and require more acid or alkali than preparations that have small amounts of solids. The data concerning the amounts of acid or alkali used, which are recorded in table 1, are valid, therefore, only for virus samples prepared as described in the first experiment. Even then, some variation may be expected with samples prepared at different times from different sets of

plants. The absence of the large amount of organic solids ordinarily present in untreated juice may be responsible for the fact that the rate of inactivation of virus in purified preparations is somewhat greater at pH 3 and somewhat less at pH 9, pH 10, and pH 11.

The fact that it was not found possible to demonstrate active virus in preparations completely inactivated at unfavorable hydrogen-ion concentrations, even after long standing at favorable reactions, indicates that such treatment does not cause inactivated virus to become active again. The reactivation experiment described by Brewer, Kraybill, Samson, and Gardner (3) could not be repeated, inasmuch as it was found impossible to inactivate virus completely at pH 8.5.

One factor, the importance of which has apparently been overlooked by many workers and that may be responsible for apparent discrepancies, is the effect of the hydrogen-ion concentration of virus preparations at time of inoculation on the infectivity of virus. It may be seen from table 3 that *Nicotiana glutinosa* was quite susceptible to virus at pH 2, pH 2.5, pH 3.0, and pH 3.5, whereas *Phaseolus vulgaris* gave only about 10 per cent. as many lesions at these high hydrogen-ion concentrations as at pH 6. However, *P. vulgaris* gave more lesions with virus at low hydrogen-ion concentrations such as pH 9.5, pH 10, and pH 10.5 than it did with virus preparations at pH 6. The actual number of lesions obtained with a virus preparation on inoculation to *P. vulgaris* may be increased or decreased remarkably by a change in the hydrogen-ion concentration of the virus preparation. For example, it may be seen from table 3 that the preparation at pH 1.5 gave an average of only 0.3 lesions per half-leaf on *P. vulgaris* or 1.1 per cent. as many as the control, whereas another portion of the same virus preparation at pH 10.0 gave 121.0 lesions per half-leaf or 485 per cent. as many as the same control, an increase of over 400 times. This increase is not due to inactivation of virus at pH 1.5, since it may be seen from table 3 that each preparation when brought to pH 6 gave about as many lesions as the untreated control. It is possible that there is a tendency for virus to aggregate at high hydrogen-ion concentrations and to become dispersed at low hydrogen-ion concentrations, and thus cause a change in the actual number of infective units present. This can not be responsible for much of the change, for, as may be seen from table 3, virus preparations at pH 2 and pH 10 still gave about the same number of lesions on *N. glutinosa* as the control. This is an indication that the number of infective units is about the same in either case. It seems likely, therefore, that most of the change is due to the effect of the hydrogen-ion concentration of the virus preparation on the cells of the plant used to measure the virus infectivity. Cells of plants of *N. glutinosa* are apparently little

affected by quite large changes in hydrogen-ion concentration, whereas cells of *P. vulgaris* plants are adversely affected, as regards susceptibility to virus, at high hydrogen-ion concentrations such as pH 1 to pH 3.5, and beneficially affected at low hydrogen-ion concentrations such as pH 9.5 to pH 10.5. In further confirmation of this point, it may be seen from table 3 that virus preparations at pH 1 and pH 11.5 gave no lesions on *P. vulgaris*, yet gave a few lesions on *N. glutinosa*. *Vigna sinensis* plants are affected by changes in the hydrogen-ion concentration of the inoculum similarly to those of *P. vulgaris*.

## SUMMARY

The rate of inactivation of tobacco-mosaic virus in diluted untreated infectious juice of *Nicotiana tabacum* L. variety Turkish held at 20° C. or −14° C. is so slow as to be negligible between pH 3 and pH 8, fairly rapid between pH 1.5 and pH 2.5 and between pH 9 and pH 10, and very rapid between pH 0.5 and pH 1.5 and between pH 11 and pH 12. The rate of inactivation of virus in purified virus preparations is similar except that the rate is somewhat greater at pH 3 and somewhat less between pH 9 and pH 11.

Reactivation of virus completely inactivated at pH 1, pH 2, pH 11, or pH 12 was not observed.

The susceptibility of plants of *Nicotiana glutinosa* L. to tobacco-mosaic virus inoculated at reactions from pH 2 to pH 10.5 did not change greatly. Plants of *Phaseolus vulgaris* L. variety Early Golden Cluster were much more susceptible to virus inoculated at reactions of from pH 9 to pH 10.5 than to virus inoculated at pH 0.5 to pH 3.

Tobacco-ring-spot and cucumber-mosaic viruses were much less stable than tobacco-mosaic virus, inactivation increasing as the hydrogen-ion concentration was increased or decreased from about pH 6.

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## LITERATURE CITED

1. ALLARD, H. A. Effect of various salts, acids, germicides, etc., upon the infectivity of the virus causing the mosaic disease of tobacco. Jour. Agr. Res. **13**: 619–637. 1918.
2. BEALE, HELEN PURDY. The serum reactions as an aid in the study of filterable viruses of plants. Contrib. Boyce Thompson Inst. **6**: 407–435. 1934.
3. BREWER, P. H., H. R. KRAYBILL, R. W. SAMSON, and M. W. GARDNER. Purification and certain properties of the virus of typical tomato mosaic. Phytopath. **20**: 943–950. 1930.
4. CALDWELL, JOHN. The physiology of virus diseases in plants. IV. The nature of

the virus agent of aucuba or yellow mosaic of tomato. Ann. Appl. Biol. **20**: 100–116. 1933.

5. CHESTER, KENNETH S. Specific quantitative neutralization of the viruses of tobacco mosaic, tobacco ring spot and cucumber mosaic by immune sera. Phytopath. **24**: 1180–1202. 1934.
6. FUKUSHI, T. On some properties of the tobacco mosaic virus. Jap. Jour. Bot. **6**: 381–392. 1933.
7. HILL, S. E. Use of an improved null instrument for glass electrode or other high resistance circuits. Science **73**: 529. 1931.
8. HOLMES, FRANCIS O. Accuracy in quantitative work with tobacco mosaic virus. Bot. Gaz. **86**: 66–81. 1928.
9. ————. Local lesions in tobacco mosaic. Bot. Gaz. **87**: 39–55. 1929.
10. JOHNSON, J., and T. J. GRANT. The properties of plant viruses from different host species. Phytopath. **22**: 741–757. 1932.
11. LOJKIN, MARY, and CARL G. VINSON. Effect of enzymes upon the infectivity of the virus of tobacco mosaic. Contrib. Boyce Thompson Inst. **3**: 147–162. 1931.
12. MACINNES, DUNCAN A., and DONALD BELCHER. A durable glass electrode. Ind. Eng. Chem., Anal. Ed., **5**: 199–200. 1933.
13. PRICE, W. C. Local lesions on bean leaves inoculated with tobacco mosaic virus. Am. Jour. Bot. **17**: 694–702. 1930.
14. SAMUEL, GEOFFREY, and T. G. BALD. On the use of the primary lesions in quantitative work with two plant viruses. Ann. Appl. Biol. **20**: 70–99. 1933.
15. STANLEY, W. M. Chemical studies on the virus of tobacco mosaic. I. Some effects of trypsin. Phytopath. **24**: 1055–1085. 1934.
16. VINSON, C. G., and A. W. PETRE. Mosaic disease of tobacco. II. Activity of the virus precipitated by lead acetate. Contrib. Boyce Thompson Inst. **3**: 131–145. 1931.

# INFLUENCE OF PHOSPHORUS AND POTASSIUM SUPPLY ON HOST SUSCEPTIBILITY TO YELLOW TOBACCO MOSAIC INFECTION[1]

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Host nutrition has long been recognized by plant pathologists as an important factor in modifying host susceptibility to parasitic infection. Up to the present time, however, little attention has been given to this factor in most plant-virus investigations. The effect of nitrogen supply on host susceptibility to infection with tobacco mosaic has already been considered in a previous paper (8). The object of the present paper is to report the results of further experiments in which the effect of phosphorus and potassium supply on the susceptibility of tobacco to virus infection was studied.

## REVIEW OF LITERATURE

A review of the plant-virus literature reveals only a very limited number of papers dealing with the effect of phosphorus and potassium supply on host susceptibility to virus infection. Schultz and Folsom (7) were among the first to consider host nutrition in connection with plant viruses. They found that the percentage of mosaic and the distinctness of mottling on Green Mountain potatoes were reduced by high nitrogen and high potassium fertilization during the first part of the season. Janssen (3), in a series of fertilizer tests with nitrogen, phosphorus, and potassium on two different types of soils, observed that the incidence of mosaic on potato was highest in plots deprived of potassium. Quanjer (5) concluded from his investigations and those of his collaborators that potassium retards the development and spread of pseudo-net-necrosis to healthy potato plants.

Ainsworth (1), in studies on permanent soil plots receiving the same treatment each year, observed that the number of tomato plants affected with tomato stripe on plots receiving a complete fertilizer was 5.0 per cent. However, when potassium was omitted from the fertilizer mixture the number of diseased plants increased to 23.3 per cent. Omission of phosphate lowered the percentage only slightly.

Volk (9), growing tomato and tobacco plants in nutrient sand cultures, obtained some interesting results on the effect of nutrition on host susceptibility to spot-mosaic virus (Fleckmosaik). He reports that susceptibility

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of tomato is not influenced noticeably by variations in the potassium supply, but is decreased when phosphorus is withheld. Tobacco plants were inoculated at the preblooming stage by injecting expressed sap under the epidermis of the lowermost leaf on each plant. The various amounts of potassium and phosphorus used exerted no change in susceptibility of tobacco to infection, susceptibility being measured by the number of plants that became diseased. These results with regard to potassium are not in accord with those found by the other workers previously mentioned.

## MATERIALS AND METHODS

In the experiments here reported, *Nicotiana tabacum* L. var. Turkish was used as the host plant. Growth was measured by determining both the green weights and the heights of representative plants in each test at time of inoculation. Only green-weight data will be presented, however, since the resultant graphs of these two growth criteria proved to be similar. Susceptibility to infection was measured by counting the number of primary lesions for each 100 pin punctures.

Tobacco seedlings were germinated in white quartz sand and grown in this medium for about 3 weeks, during which time they were kept moist with a dilute complete nutrient solution. When the seedlings had developed 3 or 4 small leaves and were about 2 cm. in height, uniform plants were selected and potted in 4-inch porous clay pots in a soil of low fertility. The pots were then placed in saucers on a bench in a greenhouse and were spaced sufficiently to prevent undue crowding of foliage. While the experiments were in progress the greenhouse temperature was held between 70° and 80° F. during the day and between 70° and 75° F. at night. The greenhouse was fumigated at weekly intervals to guard against possible insect transmission of the mosaic.

Nutrient treatments were started 3 or 4 days after the seedlings had been potted. The plants were grouped into several different treatments with 10 pots in each. Twice a week each plant received 100 cc. of a basic nutrient solution, to which the calculated amounts of phosphorus and potassium were added, as will be discussed later.

The virus used, a yellow tobacco-mosaic virus, designated by Johnson (4) as tobacco virus 6, was obtained from James Johnson several years ago. Since that time it has been carried through repeated transfer in tobacco plants. This virus was chosen as the infective agent because of the conspicuous primary lesions that it produces on tobacco. These lesions usually become visible as small, yellowish areas on the 3rd day after inoculation. They may be more easily distinguished if the leaves are first decolorized in warm alcohol and then stained in a weak potassium iodide-iodine solution, as described by Samuel (6). The primary lesions, in which starch

formation is inhibited completely, show up as clear spots against the dark background of healthy starch-filled tissue. Figure 1 shows a typical tobacco leaf after such treatment on the 8th day following inoculation.

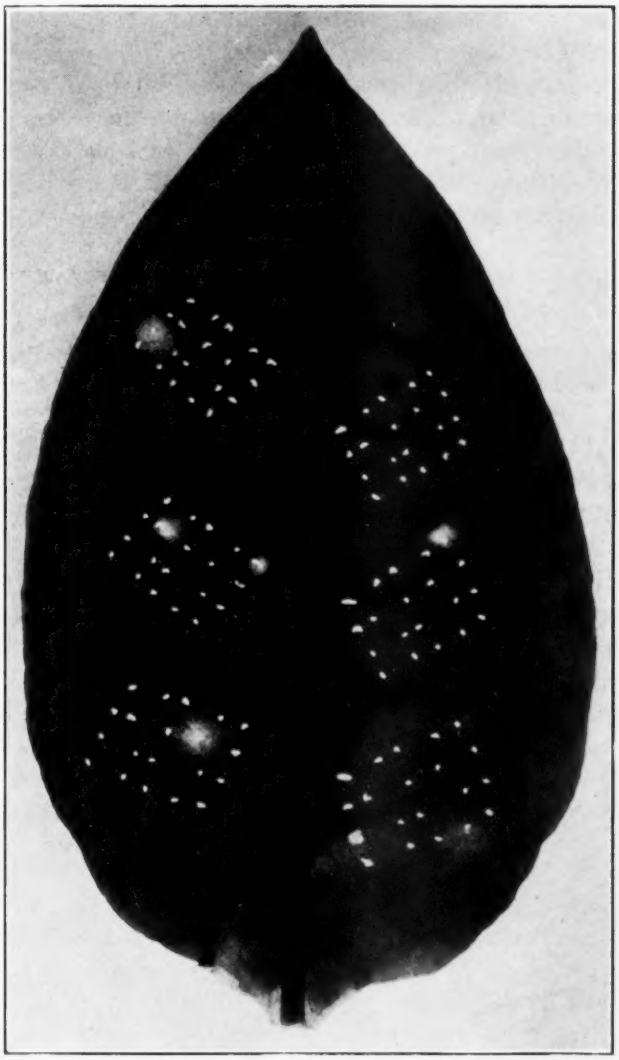

FIG. 1. Primary lesions of yellow tobacco mosaic on tobacco after the leaf has been stained with iodine. The primary lesions appear as clear areas against the dark background.

When the majority of the plants had formed 12 or 13 leaves, representative plants were inoculated. The pin-puncture method, as described

by Holmes (2), was used in order to introduce relatively uniform doses of inoculum. A set of 20 small insect pins, size No. 000, were bound between pot labels, so that 20 pricks could be made with each thrust. The number of punctures per leaf ranged from 60 to 260, depending on the size of the leaf. In these experiments only leaves in the upper and middle portion of each plant were inoculated, since data presented in an earlier paper (8) showed that results obtained from leaves on the upper and middle portion of a plant were similar to those obtained when all the leaves were inoculated. The pins were dipped into undiluted juice from a diseased plant each time before being pricked into the test plant. All experiments have been repeated one or more times.

## INFLUENCE OF PHOSPHORUS SUPPLY ON SUSCEPTIBILITY TO INFECTION WITH YELLOW TOBACCO MOSAIC

The basic nutrient solution used in the phosphorus tests was made up of a $MgSO_4$ and $CaCl_2$ solution to which nitrogen, potassium and phosphorus were added. In the 10 treatments phosphorus was added in the form of $KH_2PO_4$ and $NH_4H_2PO_4$ in doses ranging from 0 to 150 mg. of phosphorus per plant per day. The potassium concentration of all solutions was held constant at 100 mg. per plant by adding the calculated amounts of $K_2SO_4$. The nitrogen concentration was held at 31 mg. per plant by adding the required amounts of $(NH_4)_2SO_4$. After 3 weeks of nutrient treatment the plants were large enough to be inoculated.

Figure 2 shows representative plants that received 0, 5, 20, 60, and 150 mg. of phosphorus per day, respectively, for 25 days, photographed at time of inoculation. Growth was not greatly retarded even when 150 mg. of phosphorus per day were added. The color of all the plants was similar

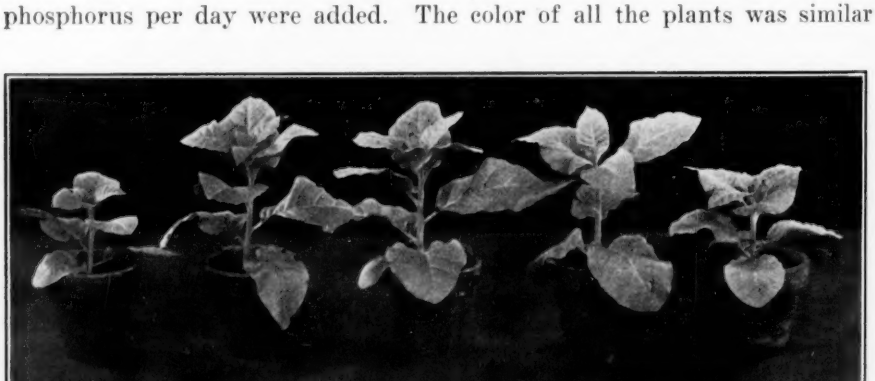

FIG. 2. Typical tobacco plants that received 0, 5, 20, 60, and 150 mg. of phosphorus per day, respectively. The middle plants were nearly equal in susceptibility.

except in the case of those receiving no phosphorus. The latter were a dark olive green.

TABLE 1.—*Effect of phosphorus supply on growth and susceptibility of tobacco to yellow tobacco mosaic*

| Phosphorus added per day (*mg.*) | Green weight at time of inoculation (*gm.*) | Number of | | |
|---|---|---|---|---|
| | | Pin punctures | Lesions | Lesions per 100 pin punctures |
| 0 | 19[a] | 8300[b] | 366[b] | 4.4 |
| 5 | 41 | 12640 | 1391 | 11.0 |
| 10 | 40 | 13600 | 1518 | 11.2 |
| 20 | 41 | 13600 | 1551 | 11.4 |
| 40 | 41 | 13600 | 1468 | 10.8 |
| 60 | 39 | 13600 | 1423 | 10.5 |
| 80 | 41 | 13600 | 1305 | 9.6 |
| 100 | 36 | 12400 | 1153 | 9.3 |
| 125 | 36 | 12400 | 1054 | 8.5 |
| 150 | 26 | 9700 | 743 | 7.7 |

[a] Average of 10 plants.

[b] Total number on 10 plants.

In table 1 are recorded the composite results of two tests on the influence of phosphorus supply on growth and susceptibility to infection. The green-weight data at time of inoculation represent the average of 10 plants for each treatment. These data show that equally good growth was produced by varying amounts of phosphorus (5–80 mg. of phosphorus per day). With larger doses of phosphorus, total growth of the plants was less. From this table it is also evident that several different doses of phosphorus gave equally high susceptibility (5–60 mg. of phosphorus per day), although susceptibility was gradually lowered when more than 20 mg. of phosphorus were added per day.

The influence of phosphorus supply on growth and susceptibility is expressed graphically in figure 3. The solid line represents total growth as measured by green weights of representative plants at time of inoculation. The broken line represents susceptibility to infection as measured by the number of lesions per 100 pin punctures. These graphs show that susceptibility was correlated directly with growth. That is to say, susceptibility increased when growth increased, and decreased when growth fell off due to excess phosphorus. It is probable that this variation in susceptibility was due primarily to variation in growth and only indirectly to the phosphorus supply.

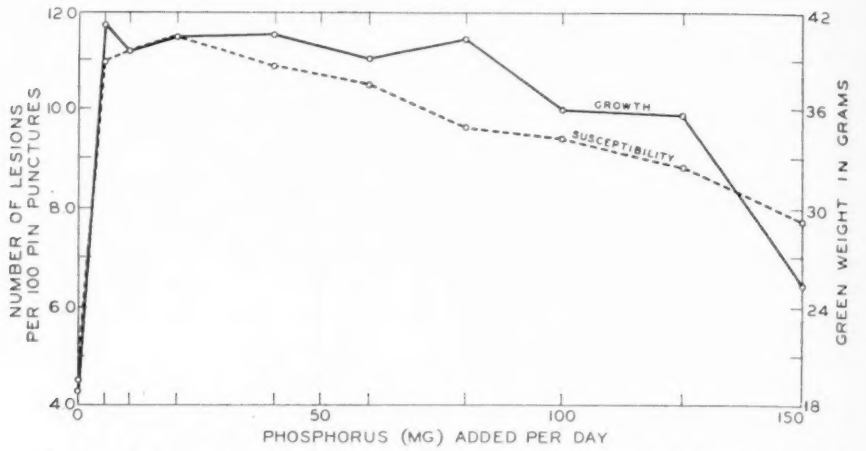


FIG. 3. Influence of phosphorus supply on growth and susceptibility of tobacco to yellow tobacco mosaic. The solid line represents green weight of plants; the broken line, the number of lesions per 100 pin punctures.

## INFLUENCE OF POTASSIUM SUPPLY ON SUSCEPTIBILITY TO INFECTION WITH YELLOW TOBACCO MOSAIC

In the potassium tests the doses received by the plants in the 11 treatments ranged from 0 to 500 mg. of potassium, in the form of $K_2SO_4$, per plant per day. The supply of the other essential elements was held constant in all treatments. Each plant received 14.6 mg. of nitrogen and 10.0 mg. of phosphorus with every feeding of potassium. Small amounts of calcium and magnesium also were added. The plants were inoculated 30 to 34 days after potassium treatments were started.

FIG. 4. Typical tobacco plants that received 0, 20, 100, 150, and 300 mg. of potassium per day, respectively, from left to right, photographed at time of inoculation. The second plant from the left was the most susceptible.

Figure 4 shows typical tobacco plants that received 0, 20, 100, 150, and 300 mg. of potassium per day, respectively, photographed at time of inoculation. No symptoms of toxicity were noticed in any of the treatments when less than 400 mg. of potassium per day were added. Above this dose, the plants became dwarfed and made very little growth. When no potassium was added, the foliage became conspicuously mottled and yellowish in color. The oldest leaves in this treatment were spotted with white necrotic areas where tissue had broken down.

The composite results of three tests on the influence of potassium supply on growth and susceptibility to infection are given in table 2. From the

TABLE 2.—*Effect of potassium supply on growth and susceptibility of tobacco to yellow tobacco mosaic*

| Potassium added per day (*mg.*) | Green weight at time of inoculation (*gm.*) | Number of | | |
|---|---|---|---|---|
| | | Pin punctures | Lesions | Lesions per 100 pin punctures |
| 0 | 15[a] | 15000[b] | 379[b] | 2.5 |
| 10 | 26 | 13100 | 585 | 4.5 |
| 20 | 26 | 19600 | 931 | 4.8 |
| 40 | 27 | 19600 | 712 | 3.6 |
| 70 | 27 | 19600 | 633 | 3.2 |
| 100 | 27 | 18640 | 644 | 3.5 |
| 150 | 24 | 18640 | 577 | 3.1 |
| 200 | 23 | 17060 | 401 | 2.4 |
| 300 | 16 | 12200 | 291 | 2.4 |
| 400 | 7 | 3140 | 48 | 1.5 |
| 500 | 4 | 1500 | 17 | 1.1 |

[a] Average of 15 plants.

[b] Total number on 15 plants.

green-weight data, which represent averages of 15 plants on each treatment, it is evident that equally good growth was produced with a wide range of potassium dosages (10–150 mg. of potassium per day). Beyond this range growth fell off gradually until, at 500 mg. per day, growth was retarded to such an extent that most of the plants died. From this table it also is apparent that susceptibility, as measured by the average number of lesions per 100 pin punctures, was highest with very low doses of potassium and decreased when the size of the dose of potassium was increased.

In figure 5 the relation between growth and susceptibility as influenced by potassium supply is expressed graphically. The solid line represents average green weights of representative plants at time of inoculation. The broken line represents the average number of lesions per 100 pin punctures. These graphs show that good growth was produced by the addition of

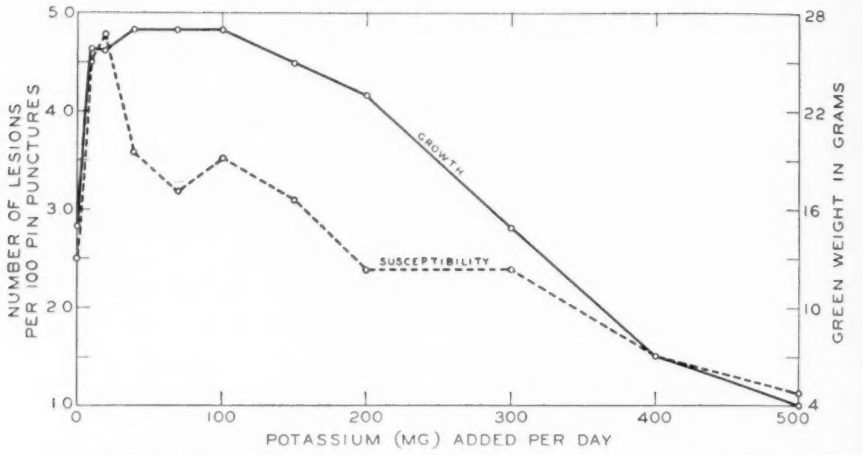


FIG. 5. Relation between growth and susceptibility of tobacco to yellow tobacco mosaic as influenced by potassium supply. The solid line represents green weight of plants; the broken line, the number of lesions per 100 pin punctures when potassium is added as indicated along the base line.

from 10 to 150 mg. of potassium per day. High susceptibility, on the other hand, was limited to very small doses of potassium, between 10 and 20 mg. per day. Below and above this restricted range, susceptibility fell off markedly. Thus, it is evident that host susceptibility to infection was influenced more by potassium than it was by growth, since doses of potassium between 20 and 150 mg. per day decreased susceptibility pronouncedly, whereas growth remained constant.

## DISCUSSION

Data obtained in this investigation indicate that phosphorus supply has apparently only an indirect influence on susceptibility of tobacco to infection with yellow tobacco mosaic, inasmuch as susceptibility and growth seem to be correlated directly with each other. Potassium supply, on the other hand, has a direct influence in changing host susceptibility to infection. When more than 20 mg. of potassium were added each day, susceptibility decreased appreciably. Growth, however, was not retarded noticeably until the addition of potassium exceeded 200 mg. per day.

These experiments do not permit the formulation of any definite explanation of the effect of potassium and phosphorus supply on host susceptibility to parasitic infection. Although it is concluded that host susceptibility has been altered by mineral nutrition, further evidence is needed to show whether certain of the mineral elements, or some substances dependent on a specific mineral nutrition for their synthesis, are the factors determining the degree of susceptibility of the host. This evidence can

be obtained only by an investigation of the physiological and morphological conditions that exist within the plant itself.

The physiological rôles of potassium and phosphorus in plants have been studied by many plant physiologists. The effect of deficiencies of these particular elements on the histological structure and organic constituents of plants also has been investigated, but the effect of excess potassium and phosphorus on plant organisms is still a matter of question. An explanation of the apparent tolerance of tobacco to large amounts of potassium and phosphorus without any visible change in growth response is difficult. It has long been known by soil scientists that vegetative and reproductive conditions of plants are not changed appreciably by the "luxury consumption" of salts of potassium, phosphorus, and other minerals. It may be that the response of tobacco to large amounts of these elements can be explained on this basis.

The conclusion that potassium decreases susceptibility in tobacco substantiates the findings of several investigators with other plant viruses, but is not in accord with those published by Volk (9), who found that potassium and phosphorus exerted no influence on the susceptibility of tobacco.

## SUMMARY

The influence of phosphorus and potassium supply on growth and susceptibility of tobacco to yellow tobacco mosaic was studied.

Plants grown in soil with various doses of potassium and phosphorus were inoculated by the pin-puncture method with yellow tobacco-mosaic virus (Johnson's tobacco virus 6). Growth was measured by green-weight determinations at time of inoculation, and susceptibility to infection by the number of lesions per 100 pin punctures.

In the phosphorus study susceptibility was correlated directly with growth. It is probable that this variation in susceptibility was due primarily to variations in growth and thus only indirectly to the phosphorus supply.

Results from the study of potassium indicate that host susceptibility was influenced more by potassium supply than it was by growth. Growth was favored by small and medium doses of potassium. Susceptibility to infection, however, was increased by very small doses, but was decreased markedly by moderately larger doses. Thus, susceptibility to infection can be lowered without retarding growth by increasing the supply of potassium within certain limits.

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LITERATURE CITED

1. AINSWORTH, G. C. Mosaic disease of the tomato. Cheshunt (England) Exp. and Res. Sta. Ann. Rept. **17** (1931): 42–43. 1932.
2. HOLMES, F. O. Accuracy in quantitative work with tobacco mosaic virus. Bot. Gaz. **86**: 66–81. 1928.
3. JANSSEN, J. J. Invloed der bemesting op de gezondheid van de aardappel. Tijdschr. Plantenz. **35**: 119–151. 1929.
4. JOHNSON, J. The classification of plant viruses. Wisconsin Agr. Exp. Sta. Res. Bul. 76. 1927.
5. QUANJER, H. M. Die Selektion der Kartoffel und der Einfluss äusserer Umstände, insbesondere der Düngung, auf das Selektionsergebnis. Ernähr. Pflanze **27**: 1–8. 1931.
6. SAMUEL, G. Some experiments on inoculating methods with plant viruses, and on local lesions. Ann. Appl. Biol. **18**: 494–507. 1931.
7. SCHULTZ, E. S., and D. FOLSOM. Transmission, variation, and control of certain degeneration diseases of Irish potatoes. Jour. Agr. Res. **25**: 43–118. 1923.
8. SPENCER, E. L. Effect of nitrogen supply on host susceptibility to virus infection. Phytopath. **25**: 178–191. 1935.
9. VOLK, A. Beiträge zum Kenntnis der Wechselbeziehungen zwischen Kulturpflanzen, ihren Parasiten und der Umwelt. IV. Einflüsse des Bodens, der Luft und des Lichtes auf die Empfänglichkeit der Pflanzen für Krankheiten. Phytopath. Zeitschr. **3**: 1–88. 1931.

# INFECCIÓN EXPERIMENTAL DEL CENTENO DE PETKUS (SECALE CEREALE V. VULGARE), POR LAS CARIES DEL TRIGO: TILLETIA TRITICI Y TILLETIA LEVIS

RAIMUNDO NIEVES, ING. AGR.[1]

(Accepted for publication February 3, 1934)

Durante 3 años, 1931, 1932 y 1933, he obtenido experimentalmente *Tilletia tritici* (Bjerk.) Wint. y *Tilletia levis* Kühn, sobre *Secale cereale* v. *vulgare*.

Solo ciertas formas fisiológicas de estos hongos, parecen capaces de atacar a este nuevo huésped, siendo la primera vez que se cita al centeno de Petkus (variedad oriunda de Alemania) como infectado por ambas Tilletias del trigo. Por otra parte, las comunicaciones sobre este género de parasitismo, son sumamente escasas.

He creido conveniente no demorar mas la comunicación de estos resultados[2] por cuanto los ensayos de 1931 y 1932, son absolutamente convincentes, en el sentido de evidenciar al centeno como huésped de dos hongos que en la literatura corriente y durante muchos años se han creido parásitos estrictos del trigo.

## ANTECEDENTES EN LA LITERATURA AGRÍCOLA

El centeno es atacado raramente por una carie específica, la *Tilletia secalis* (Cda.) Kühn, señalada para Europa desde hace muchos años (14). Sus esporos son análogos a los de *Tilletia tritici*. En Estados Unidos ha sido señalada por Kirby (12); pero tambien como de aparición muy rara. Kirby dice: "La carie del centeno (*Tilletia secalis*) ha sido encontrada una vez en New York. El centeno es muy resistente, y nunca se ha encontrado mas que unas pocas espigas infectadas. La carie del trigo comunmente encontrada en este Estado, es causada por otra especie de hongo (*T. tritici*). Si este hongo ataca al centeno es desconocido." Ignoro si en nuestro país ha sido señalada la *Tilletia secalis*, pero creo probable que no.

La aparición espontánea de la caries del trigo sobre el centeno, o su inoculación experimental, son aparentemente hechos recien establecidos. Vavilov en su clásica monografía (25) al dar la lista de los géneros

[1] Jefe de la Sección Fitotecnia del Ministerio de Agricultura de la Nación.

[2] The writer wishes to thank Dr. H. B. Humphrey for his hearty cooperation during the progress of this work and for helpful suggestions in the preparation of the manuscript.

y especies de cereales atacados por *Tilletia tritici*, consigna únicamente el género *Triticum* sp. y *Aegilops* sp. No hace referencia alguna sobre *T. levis*. Ignora al centeno como huésped de la *T. tritici*, pero lo señala como huésped de *Ustilago tritici* (Pers.) Rostr., habiéndolo encontrado el autor en el norte de Persia, cerca de Hamadan, en un campo de trigo, en el que el centeno era espontáneo. Delacroix y Maublanc (4), Voglino (26) y Stevens and Hall (24) en sus manuales de Patología Vegetal, ignoran al centeno como huésped de las Tilletias del trigo. Según Heald (9) la primera información parece pertenecer a Gaines y Stevenson (1923) quienes producen experimentalmente en Pullman (Washington), *Tilletia tritici* sobre centeno e híbridos de trigo por centeno. Johnston[3] en Kansas, infectando experimentalmente centeno con *Tilletia* sp. (probablemente *T. levis*), encuentra algunas espigas infectadas, sobre varios miles de espigas examinadas.

Bressman (1) patólogo de la Oregon Agr. Exp. Sta., inocula en 1930 los centenos Oregon Sel. 1 y Rosen, con 31 colecciones de caries. Solo la *T. tritici* f.f. 9 infectó ambos centenos. Se examinaron 4,000 espigas, en los ensayos realizados en Corvallis. En material duplicado sembrado en otro lugar de Oregon (en Pendleton), Foster Martin encuentra que ambas Tilletias infectan el centeno, pero aquí tambien la *T. tritici* f.f. 9, se manifestó la más virulenta. Bressman concluye su trabajo sugiriendo si la *T. secalis* (Cda.) Kühn, no sería una forma fisiológica de *T. tritici* y debiera ser designada como tal. Si las Tilletias del trigo tienen mas huéspedes que los citados, no creo que esté bien establecido en la literatura corriente.

Voglino (26) refiriéndose aparentemente a la *T. tritici*, dice de un modo ambiguo: "La carie (*Tilletia caries* Tulasne), infesta le pianticine di grano ed un gran numero di graminacee selvatiche." La *T. levis* la consigna como específica del trigo.

Recientemente Bressman (3), consiguió inocular experimentalmente *T. levis* sobre *Lolium multiflorum* y *Lolium perenne*, consiguiendo 0.5 a 1 por ciento de infeccion. En nuestro país no se había señalado el parasitismo espontáneo o experimental de las Tilletias del trigo sobre el centeno, hasta la aparición de mi trabajo "La caries del trigo," (15) donde trato este asunto de manera incidental. Pero esta monografía es la primera, donde considero el tema con suficiente amplitud.

## MATERIAL Y MÉTODOS

El centeno de Petkus, me fué proporcionado por el Ingº R. Bence Pieres, en 1926, quien lo recibió del Dr. Restelli, entonces Subsecretario del Ministerio de Relaciones Exteriores. Las colecciones de *Tilletia* sp. han sido recogidas por mí, en giras por las chacras, y otras solicitadas a diversos

[3] Johnston, C. O. U. S. Dept. Agr. Plant Dis. Reptr. Suppl. 62: 324. 1928.

colegas. Sobre 43 procedencias testadas en 1933, 34 las he colectado personalmente. Me fueron provistas: las colecciones 14, 15 y 17, por el Ingº Domingo Dávila; la 21 por el Ingº Santiago Boaglio; la 22 por el Agrº Williamson; la 24 por el Ingº Vicente C. Brunini; la 25, 26, 27 y 28 por el Dr. Wilhem Rudorf. Agradezco a mis distinguidos colegas su colaboración.

Todas las procedencias corresponden a La Pampa y Provincia de Buenos Aires, excepto las provistas por el Dr. Rudorf, que corresponden a Alemania. Las inoculaciones experimentales, se realizaron empolvando hasta el ennegrecimiento la semilla de los huéspedes (trigos o centeno), poco antes de la siembra, con clamidósporos frescos (del año anterior) de las *Tilletia* spp. Como es lógico, se han evitado posibles contaminaciones entre colecciones.

En cada ensayo se hicieron siembras individuales de los granos, comunmente a 7.5 × 20 cms. y a veces a 15 × 15 cms. Comunmente destinaba una parcela de 180 plantas para cada variedad. Los operadores se lavan las manos con soluciones de formol, despues de manipular cada colección. La época de siembra preferida fué a fines de Abril o principios de Mayo, pues entonces tenemos en Guatraché temperaturas óptimas para conseguir pesadas infecciones. Por otra parte la humedad del suelo y la contextura fina y friable de este último, son normalmente óptimos para la mejor germinación de las Tilletias.

Despues de la maduracióon de los trigos y centeno, se arrancan las plantas, se engavillan, etiquetan, etc. y luego se analizan. El recuento de las espigas infectadas, se hace pellizcando las espigas en dos o tres partes, a fin de poner en evidencia si tiene granos sanos o cápsulas de carbón (smut

TABLA Nº 1.—*Resultados de la infección experimental del centeno por 6 colecciones de T. tritici y 2 de T. levis, en el año 1931–32*

| Siembra: Abril 23 | Colecciones de *T. tritici* | | | | | | Colecciones de *T. levis* | | Totales |
|---|---|---|---|---|---|---|---|---|---|
| | 2 | 3 | 5 | 6 | 7 | 9 | 1 | 8 | |
| Procedencia | L.M. | Pel. | 21 | G | G | G | L. | 21 | |
| Nº espigas | 789 | 930 | 937 | 738 | 916 | 677 | 752 | 1022 | 6761 |
| Esp. infec. | — | 15 | — | 8 | — | 16 | — | 29 | 68 |
| (Por ciento) | 0 | 1.7 | 0 | 1.1 | 0 | 2.4 | 0 | 2.9 | 10 o/oo |
| Siembra: Julio 20 | | | | | | | | | |
| Nº espigas | 922 | 1089 | 1203 | 918 | 1144 | 892 | 1277 | 1222 | 8667 |
| Esp. infec. | — | — | — | 2 | — | — | — | — | 2 |
| (Por ciento) | 0 | 0 | 0 | 0.2 | 0 | 0 | 0 | 0 | 0.2 o/oo |

balls). Para el personal especializado basta generalmente ver la espiga para juzgar si está sana o enferma. Pero en los casos dudosos o con personal poco familiarizado se utiliza siempre el método del "pellizcado."

En los ensayos que consigno en las tablas N° 1 y 2, se han examinado 39.327 espigas de centeno. En la tabla 3; 53.534 espigas de trigo.

## RESULTADOS

En 1931, en un ensayo sobre especialización fisiológica de la caries del trigo (siembra temprana), en el que estudiaba la patogenia de colecciones de *Tilletia* sp. sobre 18 huéspedes diferenciales, conseguí vencer la inmunidad natural del centeno de Petkus, obteniendo 68 espigas infectadas, sobre 6.761 examinadas—10 por mil—(véase tabla n° 1). Las colecciones n° 3; 6 y 9 de *T. tritici*, y la colección n° 8 de *T. levis* infectaron el centeno.

El mismo ensayo, pero en siembra tardía, proporcionó solo 2 espigas infectadas sobre 8.667 examinadas (0.2 por mil). Solo la colección n° 6 de *T. tritici*, infectó al centeno. Fué visible en este ensayo, la influencia desfavorable de la temperatura y humedad del suelo, durante la germinación del huésped, sobre la infección final.

En 1932, en el ensayo sobre especialización fisiológica, tenía 24 colecciones de *Tilletia* spp. sobre 21 huéspedes diferenciales, y el centeno de Petkus lo incluía nuevamente por concederle gran valor diferencial. En este ensayo (véase tabla n° 2) sobre 23,780 espigas de centeno examinadas, 49 aparecieron infectadas (el 2.2 por mil). Las colecciones n° 9; 11/6; 20 y 23/3 de *T. tritici* y las n° 1; 18 y 21 de *T. levis*, infectaron el centeno, resultando inmune a las 14 colecciones restantes. Las colecciones 11/6 y 23/3 de *T. tritici*, y la 10/8 de *T. levis*, son las caries recogidas sobre el centeno infectado por las colecciones n° 6; 3 y 8 en 1931. Con las colecciones 10/8; 11/6 y 23/3, además de inocular el centeno, hice lo que puede llamarse un ensayo de inoculación inverso. Es decir, inoculé las 20 variedades de trigo huéspedes diferenciales, con las caries procedentes del centeno. Los resultados obtenidos confirmaron ampliamente la identidad de las Tilletias investigadas. Como puede verse en la tabla n° 3, se obtuvo hasta 95.5 por ciento de infección en trigos inoculados con caries procedentes del centeno. Los datos consignados en la tabla n° 3, corresponden a un total de 53.534 espigas examinadas.

En 1933, en el mismo ensayo sobre especialización fisiológica, tengo en observación 43 colecciónes de *Tilletia* spp., sobre 11 huéspedes diferenciales (8 *Triticum vulgare;* 2 *Triticum durum* y 1 centeno). Hay en este ensayo aproximadamente 80.000 plantas inoculadas. Desde hace pocos días, he podido observar la aparición de espigas de centeno, infectadas por colecciones de ambas Tilletias. Es una nueva prueba de la capacidad infectante de las Tilletias del trigo sobre el centeno. Los análisis de este material (que

TABLA Nº 2.—*Resultados de la infección experimental del centeno, por 18 colecciones de T. tritici y 6 colecciones de Tilletia levis, en el año 1932–33*

Siembra: Abril 27

| Especie | *Tilletia tritici* | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Nº colección | 2 | 3 | 4 | 5 | 6 | 7 | 9 | 11/6 | 12 | 13 | 14 | 15 | 16 | 17 | 19 | 20 | 23/3 | 24 |
| Procedencia | L. Matienze | C. Pellegrini | La Dulce | El 21 | Guatraché | Guatraché | Guatraché | Guatraché | Guatraché | Guatraché | P. Oscuro | P. Oscuro | Guatraché | Toay | Rivera | Avestruz | Guatraché | Barrow |
| Nº espigas | 1345 | 1492 | 1277 | 940 | 1095 | 1158 | 787 | 771 | 766 | 733 | 670 | 906 | 963 | 1179 | 768 | 1048 | 976 | 961 |
| Esp. infect. | — | — | — | — | — | — | 4 | 1 | — | — | — | — | — | — | — | 15 | 1 | — |
| (Por ciento) | — | — | — | — | — | — | 0.5 | 0.2 | — | — | — | — | — | — | — | 1.6 | 0.1 | — |

| | *Tilletia levis* | | | | | | Totales | Total 2 años |
|---|---|---|---|---|---|---|---|---|
| Nº | 1 | 8 | 10/8 | 18 | 21 | 22 | | |
| | Lumb | El 21 | Guatr. | Guatr. | Perga. | Pico | | |
| Nº espigas | 1416 | 815 | 769 | 1037 | 1017 | 891 | 23.780 | 39.208 |
| Esp. infect. | 13 | — | — | 12 | 3 | — | 49 | 119 |
| (Por ciento) | 0.9 | — | — | 1.2 | 0.3 | — | 2.2 o/oo | 3 o/oo |
| | | | | | | | | 39.327 |

TABLA Nº 3.—*Con los resultados obtenidos infectando 21 huéspedes diferenciales con Tilletias procedentes del centeno (colecciones 11/6 y 10/8) y las mismas Tilletias pero recogidas sobre trigo (colecciones 6 y 8). Año 1932*

| Huésped diferencial | Huésped anterior | | | | Total |
|---|---|---|---|---|---|
| | Centeno *T. tritici* 11/6 | Trigo *T. tritici* 6 | Centeno *T. levis* 10/8 | Trigo *T. levis* 8 | |
| Nº de espigas | 9.677 | 14.161 | 13.008 | 16.688 | 53.534 |
| | *Por ciento* | *Por ciento* | *Por ciento* | *Por ciento* | |
| Florence | 2.4 | 5.1 | 5.8 | 15.2 | |
| Kanred | 19.5 | 3.5 | 93.8 | 90.7 | |
| 75–26–86 | 7.4 | 1.8 | 95.5 | 89.6 | |
| Tenmarq | 22.3 | 9.6 | 90.8 | 85.5 | |
| Rubión | 0.0 | 0.2 | 8.3 | 5.7 | |
| A c d 3 | 1.1 | 0.9 | 2.0 | 1.5 | |
| 45 b | 2.9 | 9.7 | 16.5 | 14.6 | |
| 183 c 146/25 | 13.3 | 4.4 | 90.5 | 90.5 | |
| K–01–28 | 8.5 | 4.5 | 89.1 | 89.6 | |
| K–0131–26 | 8.2 | 3.1 | 91.7 | 93.4 | |
| Marquis | 49.7 | 52.1 | 74.2 | 81.5 | |
| Széckács 1055 | 1.5 | 2.5 | 61.9 | 49.5 | |
| Centeno | 0.2 | 0.0 | 0.0 | 0.0 | |
| 38 M. A. | 20.6 | 8.8 | 4.6 | 11.3 | |
| Oro C. I. 8220 | 3.3 | 1.0 | 3.1 | 8.3 | |
| Kooperatorka C. I. 8861 | 0.0 | 0.0 | 2.5 | 25.0 | |
| Wheat × Rye C. I. 8890 | 0.4 | 2.1 | 4.9 | 10.0 | |
| Bolau Selection | 0.2 | 0.0 | 0.9 | 10.3 | |
| Grand Nº 4 Selection | 0.0 | 0.2 | 4.2 | 13.0 | |
| Ioturk (Iowa Nº 1184) | — | 2.0 | 9.2 | 38.0 | |
| Minturki × (Beloglina × Buffum) C. I. 8033 | — | 9.3 | 3.0 | 13.6 | |
| Promedios | 8.5 | 5.7 | 35.8 | 39.8 | |

como es lógico deben postergarse hasta la maduración del mismo) arrojarán nuevas luces sobre este interesante caso de parasitismo.

ALGUNAS CONSIDERACIONES SOBRE LOS RESULTADOS

a. *La Especialización Fisiológica y la Resistencia Genérica.*—Examinando la tabla nº 4, resulta evidente que solo ciertas formas fisiológicas especializadas, son capaces de atacar el centeno. Quizás para los técnicos no familiarizados con estos conceptos, sea necesario aclararlos. Las f.f. esp., biotipos o razas, de un hongo, son variaciones de una misma especie, que conservando las características sistemáticas específicas, se singularizan sin embargo por otros caracteres subespecíficos. En realidad ocurre con ciertos hongos, que tienen un polimorfismo tan marcado como el que nos es habitual

TABLA Nº 4.—*Con las 3 formas fisiológicas de T. tritici y 4 f.f. de T. levis que atacaron al Secale cereale v. vulgare, en los años 1931 y 1932*

| Especie del hongo | *T. tritici* | | | | | | *T. levis* | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Colección Nº | 3 | 6 | 9 | 11/6 | 20 | 23/3 | 1 | 8 | 18 | 21 |
| a) Forma fisiológica | II | V | V | V | IX | II | I | II | III | IV |
| Procedencia | C. Pellegrini | Guatraché | Guatraché | Guatraché | Avestruz | C. Pellegrini | Lumb | El 21 | Guatraché | Pergamino |
| Por ciento infec. 1931 | 1.7 | 1.1 | 2.4 | ..... | ..... | ..... | 0 | 2.9 | ..... | ..... |
| " " " 1932 | 0 | 0 | 0.5 | 0.2 | 1.6 | 0.1 | 0.9 | 0 | 1.2 | 0.3 |

a) Las formas fisiológicas que se presentan en este cuadro, han sido aisladas por el autor en sus ensayos sobre especialización fisiológica de las caries argentinas del trigo. En 1931, sobre 6 plantas infectadas, solo una estaba totalmente infectada y tenía 29 espigas, (levis 8, f.f. II). Reducción talla 11 %.
Con tritici 6 (f.f. V), hubo reducción longitud espigas 18%.

observar en fanerógamas superiores. En *Puccinia graminis* por ejemplo, se han establecido ya, 130 f.f. esp. y su número tiende a aumentar incesantemente.

Hay distintos medios de diferenciar las f.f. de un hongo; según Stakman (23) habría 4 métodos generales de diferenciación:

1º) por su comportamiento patogénico sobre ciertos huéspedes diferenciales, (el más antiguo).
2º) por sus características culturales.
3º) por sus relaciones físico-químicas y
4º) por su morfología, (método limitado).

Desde el descubrimiento de Schroeter (22, pp. 69–70), hasta 1918, se hize labor clásica sobre especialización fisiológica de las Puccinias y del *Erisiphe graminis,* pero solo recientemente Kniep (13), tuvo la primer evidencia de la especialización de los carbunclos de los cereales (Ustilagáceas). Respecto a las Tilletias del trigo, el primero en demostrar su especialización fué Reed (17), aunque ya Faris (5) había obtenido algunos datos sugestivos. Trabajos posteriores de Sampson (21), Gaines (7), Rodenhiser y Stakman (19), Reed (18), Roemer (20), Holton (10) y recientemente Bressman (2) han confirmado de un modo indubitable los primeros hallazgos de Faris y Reed. Todos estos autores llaman formas fisiológicas en las *Tilletia* spp. a las colecciones de clamidósporos de determinada procedencia, que se singularizan por su comportamiento patogénico sobre ciertos huéspedes diferenciales. La conducta patogénica, ha ofrecido

casi siempre una regularidad remarcable, no obstante tratarse dichas colecciones de verdaderas "poblaciones."

En otros hongos las f.f. se han establecido sobre descendencias unisporidiales. En las Tilletias ciertos detalles de técnica, han hecho hasta ahora imposible adoptar el mismo criterio. (Una descendencia unisporidial, es equivalente a una "línea pura" o "pequeña especie" en el sentido de Johannsen). Es probable que en un futuro próximo, salvados dichos inconvenientes, podrá adoptarse un criterio más próximo al de las descendencias unisporidiales.

El polimorfismo de *Tilletia* sp., aparece muy marcado, y a mi juicio solo es cuestión de tiempo y trabajo establecer mas de un centenar de formas como en *Puccinia graminis*. Bressman (2), trabajando con 48 colecciones de *T. tritici* y 46 de *T. levis*, de procedencia muy variada, señala en 1931, 6 f.f. en *T. levis* y 4 en *T. tritici*.

Los otros autores citados han encontrado otras formas, pero es difícil establecer la interrelación que puede existir entre las formas de los diversos autores, porque no se han usado los mismos huéspedes diferenciales. Por otra parte, hay detalles de técnica que se oponen al uso de una serie "standard" de huéspedes en cualquier lugar geográfico, pues entre otras cosas, aún usando la misma serie, los resultados obtenidos en dos puntos geográficos son incomparables, por la influencia del medio. Como se vé, las Tilletias ofrecen complicaciones, que no valen para las Puccinias. Los autores citados, han establecido la especialización de las Tilletias, usando preferentemente el método nº 1. Alguno como Bressman, tambien ha utilizado el método nº 3. Casi todos han hecho observaciones sobre variación morfológica (método 4). Ninguno ha podido usar el método nº 2. Yo he utilizado los tres métodos viables.

En la tabla nº 5, puede verse que establezco 9 f.f. en *Tilletia tritici* y 4 f.f. en *Tilletia levis*, todas argentinas, y difundidas en el Sud de la República. En mi trabajo de 1933 aún no publicado, me ocupo con cierta extensión de esta investigación.—La severidad del método experimental, y el

TABLA Nº 5.—*Con las formas fisiológicas de T. levis y Tritici argentinas, y las colecciones de carie, que han permitido establecerlas*

| Formas fisiológicas | *Tilletia levis* | | | | *Tilletia tritici* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | II | III | IV | I | II | III | IV | V | VI | VII | VIII | IX |
| Colecciones Nº | 1 | 8 | 18 | 21 | 4 | 3 | 2 | 5 | 6 | 16 | 17 | 19 | 20 |
| | | 10 | 22? | | 12 | 23 | | 13 | 7 | | | | 24? |
| | | | | | | | | 14 | 9 | | | | |
| | | | | | | | | 15 | 11 | | | | |

TABLA N° 6.—*Mostrando el polimorfismo de las caries argentinas en solo el departamento pampeano de Guatraché*

| Formas fisiológicas | *Tilletia levis* | | *Tilletia tritici* | | | |
|---|---|---|---|---|---|---|
| | II | III | IV | V | VI | IX |
| Colecciones N° | 8; 10 | 18 | 5; 13 | 6; 7 9; 11 | 16 | 20 |

volumen del material analizado, defienden elocuentemente mis conclusiones. Solo en los ensayos de 1931 y 1932, se observaron 632,252 espigas. En un futuro próximo me será posible diferenciar mas formas. No debe olvidarse que en tal sentido hay posibilidades ilimitadas. No solo juegan su rol las mutaciones, sino que se forman nuevas combinaciones por hibridación. Asi lo demuestra Flor (6) en un trabajo reciente, en que se prueba el heterotalismo de las Tilletias, hecho supuesto, pero que dificultades de laboratorio, no habían podido evidenciar hasta ese momento.

b. *Características de la Infeccion sobre el Centeno y Variaciones Morfológicas de Orígen Patológico, del Huésped.*—Resulta evidente que el centeno, ofrece una considerable resistencia a los ataques de *Tilletia* sp. No solo es immune a gran número de colecciones (y f.f. esp.), sino que las f.f. esp. que lo atacan solo pueden infectar muy pocas espigas. Bressman ha podido obtener hasta un máximo de 6.1 por ciento de infección, con la f.f. 6 de *T. tritici*, sobre la variedad de centeno Oregon Sel. N° 1. En mis ensayos, la infección máxima fué obtenida con la forma fisiológica II de *T. levis* (Colección 8, procedencia "El 21"), en la siembra temprana del año 1931, y alcanzó solo a 2.9 por ciento. Resulta sugerente, que sobre 9 f.f. que establezco en *T. tritici*, solo 3 f.f. atacan el centero, mientras que sobre 4 f.f. que establezco en *T. levis*, todas lo atacaron. Por otra parte el material era mas abundante respecto a *T. tritici*, pues tenía 18 colecciones de esta especie y solo 6 de *T. levis*. Esta mayor patogenicidad de la *Tilletia levis* sobre el centeno, vuelve a fijar mi atención sobre la hipótesis de Bressman (1), quien al obtener el resultado que conocemos imaginó "si la *T. secalis* (Corda) Kühn, no sería una f.f. de *T. tritici*, y debía ser considerada como tal." Esta hipótesis se basa en la virulencia que observa en su f.f. n° 9, y el conocimiento, que los esporos de *T. secalis* son reticulados como los de *T. tritici*.

Basándonos en la virulencia, podemos suponer ahora: como no se ha establecido *T. secalis levis*, (es decir a clamidósporos lisos)?. Quizás no sea mas que una falla de la observación, su supuesta inexistencia. La hipótesis de Bressman es digna de concederle alguna atención experimental. No creo que se haya establecido la especificidad de *T. secalis*, y ensayado su

capacidad patógena sobre los trigos. Será necesario hacer una atenta experimentación, para aclarar definitivamente este asunto.

Volviendo a las características de la susceptibilidad en el centeno, tenemos que sobre 6 plantas infectadas en 1931, solo 1 estaba totalmente infectada, (véase tabla N° 7).

TABLA N° 7.—*Con detalles sobre las plantas de centeno infectadas en la siembra temprana de 1931*

| Tilletia | Plantas | | | Espigas[a] | | Inf. ind. |
|---|---|---|---|---|---|---|
| | Sanas | infectadas | | Sanas | infectadas | |
| | | Parcialmente | Totalmente | | | |
| | | | | | | *Por ciento* |
| *tritici* colec. 3 | 19 | 1 | – | 36 | 15 | 29.3 |
| " " 6 | 20 | 1 | – | 26 | 8 | 23.6 |
| *levis* colec. 8 | 26 | – | 1 | – | 26 | 100.0 |
| *tritici* colec. 9 | 12 | 2 | – | 112 | 15 | 11.9 |
| *tritici* colec. 6[b] | 39 | 1 | – | 13 | 2 | 13.4 |
| Totales | 116 | 5 | 1 | 187 | 66 | |
| Porcentajes | | 4.1 | 0.9 | | 26.1 | |

[a] El número de espigas corresponde unicamente a las plantas infectadas.
[b] Corresponde a la siembra tardía de 1931.

En 1931, tuvimos un "stand" (número de plantas por $m^2$.) muy reducido, por ser la semilla de centeno de algunos años atrás. Pero el macollaje (innovaciones) fué extraordinario. En 1932, tuvimos un "stand" normal, pero el recuento del material se hizo unicamente sobre las espigas. Puede observarse que sobre 122 plantas de centeno, en las parcelas atacadas, se encontró 4.1 por ciento de plantas parcialmente atacadas y sólo 0.9 por ciento totalmente atacadas. Los porcentajes de "infección individual" oscilaron entre 11.9 y 100 por ciento.

Es apreciable cierto "nanismo," es decir reducción de la longitud de la espiga. Las mediciones realizadas acusan un 18 por ciento de reducción. Por otra parte la planta totalmente infectada, por *T. levis* colec. 8, comparada con el promedio de tallas de plantas sanas del mismo lote, tambien ofreció "nanismo" aunque poco acentuado (11 por ciento). Bressman por su parte dice que no pudo verificar "nanismo" en su material. El número de "granos de caries," encontrado en las espigas infectadas ofrece un detalle de interés. En esta variedad de centeno, se encuentra una considerable "lacunosidad" en las espigas, resultando de que muchas flores no

producen semilla, por azares de la fecundación cruzada (alogamia) que es común al género Secale.

La "esterilidad parcial" de las espigas sanas contrasta con la "fertilidad total" de las espigas infectadas, en las cuales todas las flores se han convertido en granos de caries (smut balls). He verificado microscopicamento la naturaleza de los clamidósporos de ambas Tilletias y en cada caso, les ejemplares recogidos sobre centeno eran típicos. Por otra parte su tacto es untuoso y posee la característica fetidez propia de las colecciones originarias. Quizás convenga hacer presente que no todas las caries del trigo, exhalan el hedor fétido, que ha servido para caracterizar su presencia con el nombre de "carbón hediondo," "stinking smut," etc. Recientemente ha sido demostrado por bioquímicos canadienses, que dicho olor se debía a la presencia de trimetilamina en los clamidósporos. Hanna (8) cruzando Tilletias con y sin trimetilamina, probó que la presencia de la trimetilamina era un caracter transmisible por herencia que se debía a factores dominantes. Igualmente dominante es el reticulado de los clamidósporos.

## SUMMARY

Experimental infection of rye (var. Petkus) by wheat bunt: *Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kuehn.

During the years 1931–33, the author succeeded in infecting experimentally the rye variety known as Petkus with certain physiological strains of *Tilletia tritici* and *T. levis*. Preliminary studies indicated that the rare *Tilletia secalis* does not occur in Argentina. A survey of the literature yields reports of the occurrence of wheat Tilletias to a very limited extent on rye in Washington, Oregon, and Kansas.

In the experimental work in question seed grain was dusted shortly before planting time with the spores of the particular smut collection being studied. Grains (seeds) were planted separately $7.5 \times 20$ centimeters or at times $15 \times 15$ centimeters, with 180 plants to the unit plot. In 1931–32, 6 collections of *T. tritici* and 2 of *T. levis* were used, two sowings being made, April 23 and July 20. From the first planting, 68 infected spikelets were found in a total of 6,761, approximately 1 per cent; from the second planting, 2 only were infected in a total of 8,667, or .02 per cent. Three collections of *T. tritici* and one of *T. levis* produced infection.

In 1932–33, 18 collections of *T. tritici* and 6 of *T. levis* were used, plantings being made on April 27. Forty-nine infected spikelets were found in a total of 23,780 examined, or .22 per cent. Average infection for the two years was .3 per cent, or 119 infected spikelets out of a total examined 39,208. Of the smut collections used, 4 of *T. tritici* and 3 of *T. levis* produced infection.

During 1933–34, 43 collections of Tilletia were used, all collected in Argentina by the author and his colleagues with the exception of one received from Germany. Preliminary results of this series, which included 11 different hosts, indicate further positive results, but definite figures await maturity of the crop.

In connection with these infection studies, the author has established by the differential host method 9 physiological forms of *T. tritici* and 4 of *T. levis*. Of the forms of *T. tritici*, only 3 were found to infect rye.

ESTACIÓN EXPERIMENTAL DE GUATRACHÉ.
DIRECCIÓN GENERAL DE AGRICULTURA.
REPÚBLICA ARGENTINA.

LITERATURA CITADA

1. BRESSMAN, E. N. Rye infected with bunt of wheat. Phytopath. **21**: 437–440. 1931.
2. ———. Varietal resistance, physiologic specialization, and inheritance studies in bunt of wheat. Oregon Agr. Exp. Sta. Bul. 281. 1931.
3. ———. Lolium infected with bunt of wheat. Phytopath. **22**: 865–866. 1932.
4. DELACROIX, G., and A. MAUBLANC. Enfermedades parasitarias de las plantas cultivadas. P. Salvat. Barcelona. 1919.
5. FARIS, J. A. Factors influencing the infection of wheat by *Tilletia tritici* and *Tilletia levis*. Mycologia **16**: 259–282. 1924.
6. FLOR, H. H. Heterothallism and hybridization in *Tilletia tritici* and *Tilletia levis*. Jour. Agr. Res. **44**: 49–58. 1932.
7. GAINES, E. F. New physiologic forms of *Tilletia levis* and *T. tritici*. Phytopath. **18**: 579–588. 1928.
8. HANNA, W. F. The odor of bunt spores. Phytopath. **22**: 978–979. 1932.
9. HEALD, F. D. Manual of plant diseases. 891 pp. McGraw-Hill Book Company, Inc. New York. 1926.
10. HOLTON, C. S. A probable explanation of recent epidemics of bunt in durum wheats. Phytopath. **20**: 353–357. 1930.
11. ———. Differences in size and shape of smut balls of two physiologic forms of *Tilletia tritici*. Phytopath. **23**: 16. 1933.
12. KIRBY, R. S. Diseases of small grains. Cornell Univ. Col. Agr. Ext. Bul. 157. 1927.
13. KNIEP, H. Untersuchungen ueber den Antherenbrand (*Ustilago violacea* Pers.) Ein Beitrag zum Sexualitätsproblem. Zeitschr. Bot. **11**: 257–284. 1919.
14. KÜHN, J. *Tilletia Secalis*, eine Kornbrandform des Roggens. Bot. Ztg. **34**: 470–471. 1876.
15. NIEVES, R. La caries o carbón hediondo del trigo. Bol. Mens. Min. Agr. Argentina **32**: 397–411. 1933.
16. ———. Estudios sobre la especialización fisiológica de las caries del trigo (Tilletia tritici y Tilletia levis) en la Argentina. En Memoria Anual de la Est. Exp. de Guatraché. Año 1932. 1–108. 1933.
17. REED, G. M. Varietal susceptibility of wheat to *Tilletia laevis* Kühn. Phytopath. **14**: 437–450. 1924.

18. ————. Physiologic races of bunt of wheat. Amer. Jour. Bot. **15**: 157–170. 1928.

19. RODENHISER, H. A., and E. C. STAKMAN. Physiologic specialization in *Tilletia levis* and *Tilletia tritici*. Phytopath. **17**: 247–253. 1927.

20. ROEMER, THEODOR. Gibt es Biologische Typen von Steinbrand (*Tilletia tritici*) des Weizens? Kühn Archiv. **19**: 1–10. 1928.

21. SAMPSON, K. The relative resistance of wheat varieties to bunt (*Tilletia tritici*). Welsh Jour. Agr. **3**: 180–196. 1927.

22. SCHROETER, J. Entwicklungsgeschichte einiger Rostpilze. Beiträge zur Biologie der Pflanzen. **3**: 51–93. 1879.

23. STAKMAN, E. C. Racial specialization in plant disease fungi. *In* Lectures on plant pathology and physiology in relation to man. Mayo Foundation Lectures 1926–1927. 207 pp. W. P. Saunders, Co., Philadelphia. [1928]

24. STEVENS, F. L., and J. G. HALL. Diseases of economic plants. The Macmillan Co. New York. 1926.

25. VAVILOV, N. I. (Immunity of plants to infectious diseases.) Reprinted from Ann. Acad. Agron. Petrov., Moscow. 1918.

26. VOGLINO, P. Patologia Vegetale. 287 pp. Nuova Enc. Agr. Italiana, Parte Prima. Unione Tip. Editrice Torinese, Torino. 1924.

# NOMENCLATURE OF THE TOBACCO DOWNY MILDEW FUNGUS

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For many years the organism causing the tobacco downy mildew or blue mold was called *Peronospora hyoscyami* deBary. Recently the authors, as well as various other workers, have recognized that this name is incorrect because the tobacco downy-mildew fungus does not infect *Hyoscyamus niger*. The question has been raised as to whether this fungus should be given a new name, or should be referred to some other species already described. This question has been decided both ways in recent publications by Adam (1) and by Wolf (7), but in each case the conclusions were drawn without the author's having had access to the type material of *P. nicotianae* Speg., as collected by Spegazzini. The writers have been able to secure such type material from the Spegazzini herbarium.[1] Also it has been assumed that the only available description of the Spegazzini species was a very brief one published in 1891 (4) and listed in Saccardo (3). It has been discovered that a later publication was issued in Spanish in 1898 (5) and republished in Portuguese (6). This gives a very full account of both fungus and disease. It is proposed to consider the question of nomenclature in the light of the more complete information now available.

The Spegazzini herbarium material consisted of 4 collections, dated 1888, 1896, 1897, and 1899. The 1896 collection showed conidiophores and conidia and the other 3 showed oospores. The material was cleared in lactic acid and mounted in lacto-phenol for study.

Adam (1) lists 5 points of difference between *Peronospora nicotianae* and the Australian organism for which he proposes the name *P. tabacina*. Considering these points in detail, he states, "(1) The ultimate branchlets of *P. nicotianae* are uniformly longer and more acutely pointed than are those of *P. tabacina*." His measurements are from 8 μ, or less, to 14 μ. Average measurements[2] from collections made in the United States ranged from 8.35 μ to 11.6 μ. Similar averages for the 1896 Spegazzini collections were 9.72 μ to 13.34 μ. The difference, if it exists, is not great. Adam (2)

[1] The writers wish to express their deep appreciation of Dr. Juan B. Marchionatto, Chief of the Laboratory of Phytopathology of the Ministry of Agriculture, Buenos Aires, Argentina, for courtesies extended and to Dr. Maria M. Job, Museum of the University of La Plata, Argentina, for her kindness in lending specimens from the Spegazzini herbarium without which this paper could not have been prepared.

[2] These measurements were determined as follows: Ten measurements were made in a given microscopic field and averaged; from 5 to 10 sets of readings were made for each factor studied and the final figures given in this paper represent the range of the 5-to-10 averages thus obtained.

finds that, "The conidia of *P. nicotianae* (19 μ × 10 μ) are smaller than those of *P. tabacina* (22 μ × 17 μ)." Here the greatest difference is in breadth. The average breadths of United States collections ranged from 12.45 μ to 16.46 μ. The average of the Spegazzini conidia was from 16.72 to 17.44 μ (Fig. 1). Thus, instead of being smaller, the writers actually

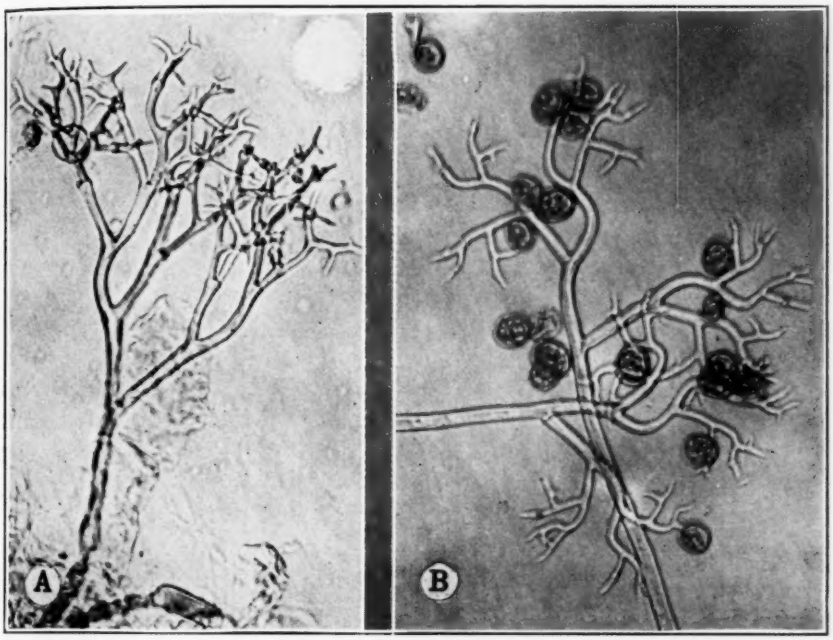


FIG. 1. A. Mature conidiophore of *Peronospora nicotianae* Speg. from material collected by Spegazzini, 1896. × 300. B. Mature conidiophores of *Peronospora tabacina* Adam from material collected in Maryland, 1933. × 300.

found the Spegazzini conidia larger. However, Angell and Hill (2) have shown that size of both conidia and conidiophores is subject to great variation under different conditions; hence it is concluded that the differences cited by Adam are without significance.

Concerning oospores, Adam points to the following: "(3) The diameter of the oospores of *Peronospora nicotianae* (50–70 μ) is much greater than that of *P. tabacina* (35–60 μ, average 46 μ)." Oospore material collected in the United States gave average measurements ranging from 32–40 μ. The Spegazzini collections gave similar averages from 62 to 78 μ. There was no question but that the Spegazzini oospores were much larger. Regarding color of oospores Adam states, "(4) The color of the oospores of *P. nicotianae*, is golden brown, that of *P. tabacina* dark brown." This color difference was found to be very striking and as stated. The marked differ-

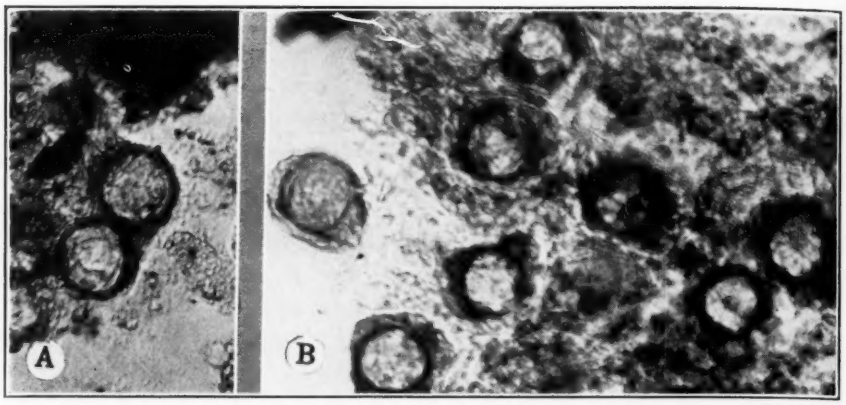


FIG. 2. Oospores of *Peronospora tabacina* Adam. ×300. A. From material supplied by F. Wolf. B. From material collected at Oxford, N. C., 1934.

ence in wall pattern also is noted by Adam. Figures 2 and 3 show the comparative size of the oospores of the two species and illustrate the characteristic wall pattern of *P. nicotianae*. It should be noted here, however, that Spegazzini did not germinate his oospores and produce the disease, nor has this been done with the oospores that the present writers and others have associated with our tobacco mildew fungus.

The grounds advanced by Wolf for believing our fungus and *Peronospora nicotianae* to be identical are now considered. He points out that the differences in size of conidia and conidiophores have no great significance. Regarding size of oospores he notes that "the size recorded by Spegazzini is 50 to 80 μ and ours 45 to 75 μ" but makes no comment as to color or wall pattern. This size, 45 to 75 μ, approaches the Spegazzini measurements closely, but collections of the present writers in the United States do not show such large oospores. The oospores on a slide kindly supplied by Dr. Wolf averaged 39.49 μ and were of the usual type.

The extensive account left by Spegazzini (5) represented a study that continued from 1888 to 1898, and his first description of the species, published in 1891, is both modified and greatly extended in this later publication. He notes that, "the disease appears first in the form of small, more or less rounded spots that are visible to the naked eye, even at a distance. These spots enlarge irregularly and by degrees until they invade not only one, but all of the leaves of the plant extending in many cases as far as the stalk; they are yellowish, more or less pronounced, and very plainly visible; the portion of the plant invaded shows a very slight increase in thickness, at the same time becoming brittle to the point of easily breaking if bent. In addition, the leaves are seen to be slightly convex, the convexity being turned toward the upper part; the stems in the diseased plants also are

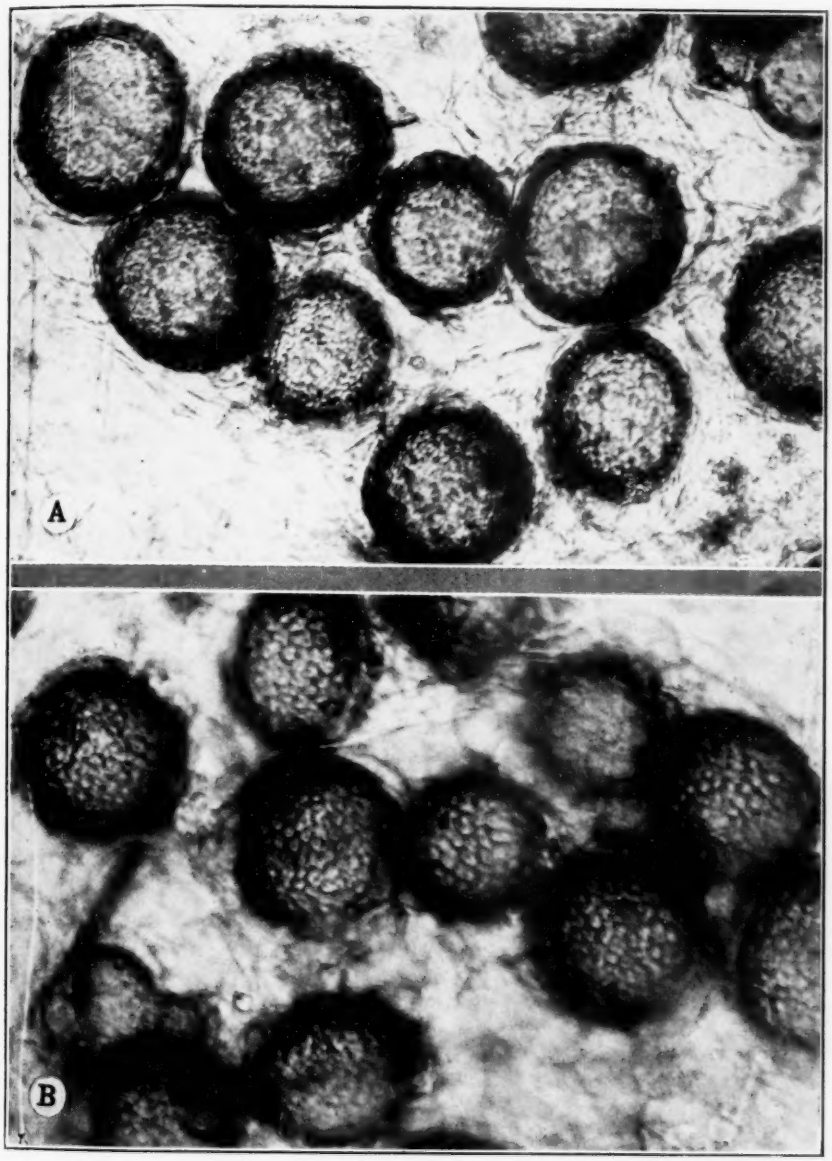


FIG. 3. Oospores of *Peronospora nicotianae* Speg. from material collected by Spegazzini, 1897. ×300. A. Focused to show size and envelope. B. Focused to show pattern of epispore.

yellow, succulent, and brittle, and have a great tendency to fasciation; it is not uncommon to see branches that, instead of following a straight line, are shaped more or less like a fish hook. The infection always begins with the lower leaves, especially with those lying on the ground and gradually extends to the stalk and upper leaves." He also mentions the downy growth of conidiophores on the lower leaf surface. Regarding conidiophores and conidia, it is stated that, "These branches are erect and straight, rising $\frac{1}{2}$ to $1\frac{1}{2}$ mm. in height, are almost cylindrical and have a thickness at their base almost three times that of the internal hyphae from which they are derived, measuring 10–12 microns in diameter, while the diameter of the upper part is less. The conidia or reproductive bodies produced at the ends of these branches are elliptical or slightly oval in shape, obtuse at both ends, and perfectly smooth. They sometimes have a very small tubercle at the lower end where they are attached to the fruiting branches. Their size is variable, measuring 15–25 μ in length and 8–14 μ in width. . . . The protoplasm with which they are filled is always colorless and consists of a very large number of granules each of which changes into a zoospore when the conidia are kept some time in water." Concerning oospores he says, "These oospores develop within the parenchyma cells themselves, from which they completely absorb the nutrient substance, provoking at the same time hypertrophy of the cell wall. A kind of chitinized covering or colorless capsule is formed. This is globular, or more or less obtusely angular, 80–100 μ in diameter, with walls having a thickness varying from 10–15 μ. It is rigid and completely smooth. On the inside of each cell there is one globular oospore, 50–80 μ in diameter, at first of a yellowish gray color, later becoming reddish gray and more or less dark or opaque owing to the thick wall of the chitinized epispore which shows a well defined design, very similar to a bee's honey comb."

"This species very closely resembles *Peronospora hyoscyami* of de Bary with which, however, I do not identify it because it lives on a different plant and because the oospores of the latter are not known." As to pathogenicity, Spegazzini reports that he collected seed of available wild species of Nicotiana, as well as cultivated varieties, and all were severely attacked by the disease. Before coming to a conclusion as to whether this mildew described by Spegazzini is the same as the disease that occurs in the United States and Australia attempts were made to obtain fresh material from the Argentine. We were advised, however, that this disease has not been observed there for at least the past 10 years.

Considering the statements in the Spegazzini publication, 2 points indicate that *Peronospora nicotianae* and our fungus may be the same. Both attack numerous species of Nicotiana and it seems unlikely that there should be two Peronosporas of such similar pathogenicity. Also, Spegazzini noted

a strong resemblance between his species and *P. hyoscyami*, and our tobacco downy-mildew fungus also resembles *P. hyoscyami*. However, in light of the fact that comparatively few species of Peronospora were known at that time, and that most conidial stages in this genus resemble each other, no importance can be attached to this observation. There is very little additional positive evidence. The description of disease symptoms does not fit particularly with those we are accustomed to seeing, except in a general way as would be true of any downy mildew. The description of oospores emphasizes again that the oospores associated with *P. nicotianae* are very different from those of our tobacco mildew. There are no significant differences as to general characters of conidiophores and conidia, but Spegazzini states that his conidia germinated indirectly with the formation of zoospores. Since at the time this was written he had had the organism under study for years, and the conidia germinate readily in water drops, it seems likely that he would have observed this germination many times. The conidia of our mildew fungus have been germinated under a wide variety of conditions and have never been observed to form zoospores. Consequently, use of the name *P. nicotianae* would necessitate the assumption that Spegazzini was mistaken in his description of both the oospore and conidial stages. While this is possible, it is possible also that a fungus fitting his description exists in South America, more especially since the writers have obtained no positive evidence that tobacco growers there have been troubled by a mildew in recent years, or that the disease described by Spegazzini was ever a crop problem.

In view of this uncertainty, it would appear inadvisable to apply the name *Peronospora nicotianae* to our fungus. *P. tabacina*, as described by Adam, on the other hand, fits our organism in all essential particulars; hence, the use of this name is recommended.

BUREAU OF PLANT INDUSTRY.

LITERATURE CITED

1. ADAM, D. B. Blue mould of tobacco. Jour. Dept. Agr. Victoria **31**: 412–416. 1933. (Reprint pp. 1–5. 1933).
2. ANGELL, H. R., and A. V. HILL. Downy mildew (blue mould) of tobacco in Australia. Counc. Sci. Ind. Res. Australia Bull. 65. 1932.
3. SACCARDO, P. A. Sylloge Fungorum **11**: 243. 1895.
4. SPEGAZZINI, C. Phycomyceteae Argentinae. Rev. Argentina Hist. Nat. **1**: 36–37. 1891. (Reprint pp. 11–12. 1891).
5. ———. Sobre una neuva enfermedad del tobaco y el polvillo de la alfalfa. Oficina Quimico-Agric. Prov. Buenos Aires Bol. 4. 1898.
6. ———. Sobre una nova molestia do fumo. A Lavoura **3**: 18–20, 74–79. 1899.
7. WOLF, F. A., L. F. DIXON, RUTH MCLEAN, and F. R. DARKIS. Downy mildew of tobacco. Phytopath. **24**: 337–363. 1934.

## PHYTOPATHOLOGICAL NOTE

*Comments on the Article*[1] *by A. Kozlowski on "Little Leaf or Rosette of Fruit Trees in California."*—The recent article by Dr. Kozlowski in this journal, while not a contribution from the University of California, and not designated as such, may confuse some readers of previous reports on the little-leaf disease in California, by Chandler, Hoagland, and Hibbard. We therefore desire to offer the following comments:

Dr. Kozlowski was formerly employed by this institution, in a temporary capacity, to assist in certain bacteriological studies on soils producing the little-leaf disease. On his own initiative Dr. Kozlowski has undertaken to interpret certain phenomena of soil science and plant pathology which fall outside his special field of knowledge. The conclusions reached are founded on a misinterpretation of certain limited observations made in the course of the general investigation and are irrelevant or inconsistent with well established facts. Dr. Kozlowski omits all reference to the most fundamental aspect of the experiments on little leaf; namely, the specific function of zinc, which clearly is not merely that of a fungicide. The views of Dr. Kozlowski should not be considered as in any way related to our own. —W. H. Chandler, D. R. Hoagland.

[1] Kozlowski, A. Little leaf or rosette of fruit trees in California. Phytopath. 25: 275–278. 1935.

## BOOK REVIEWS

*Root Rots and Degeneration of the Strawberry*

Several articles on strawberry diseases in England and Canada have appeared recently. Papers[1] by Akenhead, Harris, Berkeley, and Massee review "degeneration" in England, while Hildebrand[2] and Truscott,[3] following work by Walker and Berkeley, report on extensive studies of root rots in Canada. The complexity of the root-rot problem was indicated by Walker when he isolated 39 different organisms from diseased roots and crowns in 1927 and considered *Ramularia* sp. and *Fusarium* sp. as important causal organisms, the former being the more serious. Hildebrand, in his recent paper, reports isolating 20 different genera from 125 healthy-appearing young roots. Truscott lists as primary parasites species of Pythium, Fusarium, Alternaria, Ramularia, Rhizoctonia, Verticillium, Cylindrocladium, and Asterocystis, and notes the frequent occurrence of both a Plasmodiophoraceous fungus and a Phycomycetous mycorrhizal fungus. Hildebrand noted the almost universal presence of 2 so-called endotrophic mycorrhizal fungi, one of which was a Rhizoctonia type.

In the English articles Berkeley simply summarizes the literature on strawberry root diseases throughout the world and concludes that root rot is wide-spread and important and that, although the severity of root rot may be increased by several conditions, it may be the direct result of fungus attack and may occur in any type of soil and in plantations growing under the best cultural conditions. He already has found a *Coniothyrium* sp. associated with root rot in England.

The causal agents of root rot, as recorded in the literature noted by Berkeley, are Rhizoctonia in Michigan, Washington, New York, Oregon, Utah, Australia, and France; *Leptosphaeria coniothyrium* and *Pezizella lythri* in Michigan; *Ramularia* sp. in Canada; *Fusarium* sp. in Mississippi; *Pythium* sp. in Australia, France, Scotland, and Louisiana; *Diplodina* sp. in England, a sterile fungus in the Southern States; and a number of the Fungi Imperfecti in Scotland. He notes also Verticillium wilt in California; the Lanarkshire disease caused by an endotrophic mycorrhiza in

[1] Akenhead, D., R. V. Harris, G. H. Berkeley, and A. M. Massee. The "degeneration" of the strawberry. I.—The pomological aspect. II.—Virus. III.—Root rot. IV.—Insects and other animals. Imp. Bur. Fruit Production, East Malling, Eng., Tech. Commun. 5. 1934.

[2] Hildebrand, A. A. Recent observations on strawberry root rot in the Niagara Peninsula. Canad. Jour. Res. **11**: 18–31. 1934.

[3] Truscott, J. H. L. Fungous root rots of the strawberry. Canad. Jour. Res. **11**: 1–17. 1934.

England; a crown rot caused by *Colletotrichum fragariae* in England; a root rot in Florida caused by *Sclerotium rolfsii;* and 3 bacterial organisms producing decay of roots in Florida.

Such an array of root parasites is enough to make both the strawberry grower and strawberry runner squirm! Even the relatively definite work by Zeller, by Thomas, and by Strong and Strong in sections of the United States where a single organism, or at most 2 organisms were the important ones, leaves many unsettled problems. Control measures recommended are *setting clean plants in clean soil,* but practical directions for obeying those words are a long, long way off. Possibly conditions in Canada and England are worse, or rather more complicated than in some sections, but we suspect they are not more so than in other sections of the United States.

"Degeneration" of the strawberry in England was considered by Berkeley in relation to root rots; but, by Akenhead, in relation to the importance of vigorous runner plants, the importance of early planting, and mixtures of varieties that are apparently much more common in England than in the United States. Degeneration was considered by Harris in relation to strawberry viroses, one of which seems to be wide-spread in England. He states that experiments are in progress to determine the vectors of the "yellow-edge" virus, to study strain and varietal susceptibility, and to originate virus-resistant varieties. He also states that evidence has been obtained that certain of the clone races are more susceptible than others to outside infection.

Massee reviewed the work on the bud nema and considered that there is ample evidence that the eelworm factor may be important. In reviewing the work on aphids he states that many plantations degenerate even though aphids do not occur in them. The strawberry tarsonemid mite is found in most parts of the British Isles, and differences in varietal susceptibility were noted. This mite is being tested as a vector of the yellow-edge virus. In the 4 papers on degeneration of the strawberry, 161 references are listed. Hildebrand lists 13 and Truscott 25 papers.

Comparing conditions in England with those in the United States, Central California and the Pacific Northwest have virus problems as serious as those in England. However, more is known of the virus troubles on the Pacific Coast than of those in England. In the Eastern States the crinkle virus of the Northwest has been seen several times but is apparently not wide-spread. An apparent virus was observed by the reviewer in New England and in Maryland in 1934 in a few fields. Either the yellow-edge and "xanthosis" viroses are absent in the Eastern States or the varieties grown are resistant to them or mask their effects. Certainly through the past decades there has been opportunity for their introduction from Europe and the Pacific Coast. Possibly eastern varieties, being somewhat resistant,

do not have marked symptoms but slowly succumb. It seems hardly possible that root rot is so serious in the United States on the whole as it is in England and in Ontario, Canada. Resistant varieties, more suitable soils, a more southern latitude and climate, and recognition of root injury due to winter injury (Roberts), to ammonium sulphate (Lineberry), and to root aphis may be contributing factors. Certainly we may expect a stimulus to research on strawberry diseases in this country following these excellent papers.

Another paper by Berkeley and Lander-Thomson,[4] received after the above was written, reports proof of the pathogenicity and also the identification of 5 species of soil fungi, namely, *Coniothyrium fuckelii* Sacc., *Hainesia lythri* (Desm.) v. Höhn., *Cylindrocarpon radicicola* Wr., *Fusarium orthoceras* App. and Wr., and *Pachybasium candidum* Sacc. Of the 5, *Fusarium orthoceras* and *Coniothyrium fuckelii* appeared most virulent and the most important parasites, although *Pachybasium candidum*, a less common fungus, was very virulent. The symptoms of all five were said to be similar in all respects to those given by Strong and Strong for *Coniothyrium fuckelii* in Michigan. The *Ramularia* sp. reported by Walker was considered identical with the *Cylindrocarpon radicicola* Wr. The Cylindrocarpon and Fusarium fungi have been found on raspberry roots as well. They conclude that root rot may be due to any or all 5 fungi. However, there seemed to be seasonal and regional distribution of the different root rots. This paper adds much to our understanding of these very difficult troubles and also indicates how difficult (and important) adequate research on root rots may be.—GEORGE M. DARROW, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Washington, D. C.

*Paul Sorauer.* Handbuch der Pflanzenkrankheiten. Erster Band. Die Nichtparasitären und Virus-Krankheiten. Zweiter Teil. Sechste, neubearbeitete Auflage. Herausgegeben von Dr. O. Appel. Pp. VIII × 553. Paul Parey, Berlin. 1934. 46.00 Gold Marks.

Volume I of the sixth edition of Sorauer's Manual deals with non-parasitic and virus diseases. It is issued in two parts of approximately 500 pages each. The first part was published a little less than a year ago and was reviewed in volume 24 (p. 427–430) of this journal; the second part appeared more recently and will be discussed in the succeeding pages.

The book is in every respect a worthy companion to the introductory volume and the subject matter climaxes in a most interesting account on virus diseases. There are six main divisions:

[4] Berkeley, G. H., and I. Lander-Thomson. Root rots of strawberry in Britain. The "black lesion" type of strawberry root rot. Jour. Pom. & Hort. Sci. 12: 222–246. 1934.

Chapter 4. Diseases conditioned by internal causes. P. 1–80.
" 5. Unfavorable soil condition as cause of disease. P. 80–168.
" 6. Wounds. P. 168–240.
" 7. Smoke injuries. P. 243–310.
" 8. Injuries caused by factory wastes and sewage. P. 310–329.
" 9. Virus diseases. P. 329–512.

The chapter on plant diseases conditioned by internal factors is divided into developmental and functional disturbances during early embryogeny, the vegetative, and the reproductive period. The viewpoint of treatment is that of the geneticist. Developmental disturbances during the vegetative period deal primarily with the genetic setup responsible for dwarfing, atrophy, or loss of organs, chlorophyll defects, and necrosis. Disturbances during the reproductive period are listed as: failure of flower development, premature abscission of flowers, abortion of floral parts, notably pistils and stamens.

Chapter 5, on unfavorable soil conditions as cause of disease, comprises approximately 90 pages. It deals with both the physical and the chemical aspect of such conditions. Unfavorable physical soil states are conditioned by abnormal soil structure and an unbalanced water, air, and temperature economy. Soil loses its friability primarily through losses of electrolytes and soil colloids. A prerequisite for such a change is a humid climate; on the other hand, in arid regions with predominately unilateral water movement, there may be even an accumulation of salts in the top layers instead of a leaching. A sufficient water supply is in most cases the deciding factor for normal growth. Most growth disturbances are caused directly or indirectly by an abnormal shift in the water balance of the soil. Water deficiency manifests itself first of all in wilting, but it may produce disease symptoms such as white-spike disease of grasses, crown gall of beets, stippin apples, etc.

Unfavorable chemical changes greatly affect plant nutrition, soil reaction and are largely responsible for the accumulation of harmful substances. Numerous examples might be cited, *i.e.*, boron deficiency, responsible for heart rot of beets, and magnesium deficiency for "sand drown" of tobacco and corn. The subject of Bodenmüdigkeit—soil sickness—is treated in great detail and the various hypotheses set forth in explanation are critically examined.

The chapter on wounds is treated from the following aspects:

1. Cause of wounding: precipitation in form of rain, snow, and hail, wind, lightning, fire, excessive heat or cold and injuries caused by animal and man.

2. Reaction of plants to wounding: functional disturbances, wound heal-

ing, wound hormones; formation of wound gum and tyloses; genesis of wound cork, callus and gnarls in wood.

3. Cell and tissue regeneration following wounding; loss of roots and shoots and their restoration in woody and herbaceous plants.

4. The effect of wounding on the general development of plants.

Chapter 7 on smoke injuries deals with the toxic effect of certain chemicals such as sulphurous and sulphuric acid, chlorine, hydrochloric acid, illuminating gas, nitric acid, ammonia, asphalt fumes, etc., on the normal development of plants. The effect may be either acute or chronic and then usually invisible, manifesting itself in a gradually lowered performance. Chronic injuries, if prolonged, may become acute and then diagnosable.

Chapter 8, on factory wastes and sewage follows the same general treatment as the preceding chapter. Preventive treatments consisting in the purification and precipitation of the harmful ingredients of factory affluents are suggested.

Of greatest interest, at least to the reviewer, is the chapter on virus diseases. It comprises about 200 pages of text and 40 illustrations. But even at that the treatment of many questions is all too short and the illustrations often insufficient. One notices especially the lack of good and clear drawings in the section dealing with cytological and anatomical disturbances incident to virus infection.

The chapter on virus diseases is divided into two parts. The first part deals with the general concept of virus diseases, their aetiology and symptomology. This account is followed by a study of disease transmission, the progress of the disease and its dependence on external factors; resistance, behavior of virus *in vitro*, classification of viruses and disease control. The second part deals with specific virus diseases of solanaceous and other plants. The section on the behavior of virus in the plant body deals with the nature of infection, the movement of virus in the plant body, the rapidity of this movement, and the concept of disease recovery.

Although not as amply illustrated, the second part of Volume I is withal a worthy companion to the first part. Its wealth of information and clarity of treatment make it profitable reading suitable, however, only in small doses. The language is simple and there is a gratifying lack of involved sentences; the technical terms are not always strictly German but of Latin or Greek derivation. The text is printed on exceptionally good paper and the binding assures greatest durability.—ERNST ARTSCHWAGER, U. S. Department of Agriculture, Bureau of Plant Industry, Sugar Plants, Washington, D. C.

# REPORT OF THE TWENTY-SIXTH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

## THE PITTSBURGH MEETING

The twenty-sixth annual meeting of The American Phytopathological Society was held in Pittsburgh from December 27 to 29, 1934. The Cathedral of Learning, University of Pittsburgh, provided session rooms. Approximately 175 members were present and 110 papers were presented. In addition to regular meetings of the Society, joint sessions were held with Section G, and with the Mycological Society of America. The annual conference on Extension Work brought out much worth-while discussion with regard to the importance of close coordination between research and extension programs.

Abstracts of the papers on the program of this meeting were printed in the January number of PHYTOPATHOLOGY.

## OFFICERS AND REPRESENTATIVES

The following officers and representatives were chosen:

*President,* H. T. Güssow, Central Experimental Farm, Ottawa, Ontario, Canada.

*Vice-President,* F. C. Meier, U. S. Department of Agriculture, Washington, D. C.

*Secretary* (three years), H. P. Barss, U. S. Department of Agriculture, Washington, D. C.

*Treasurer and Business Manager* (three years), H. A. Edson, U. S. Department of Agriculture, Washington, D. C.

*Editor in Chief* (three years), H. B. Humphrey, U. S. Department of Agriculture, Washington, D. C.

*Councilor* (two years), J. C. Walker, University of Wisconsin, Madison, Wisconsin.

*Advertising Manager* (one year), R. S. Kirby, Pennsylvania State College, State College, Pa.

*Representatives on the Council of the A. A. A. S.* (one year), N. E. Stevens, U. S. Department of Agriculture, Washington, D. C., and H. S. Cunningham, Long Island Vegetable Research Farm, Riverhead, N. Y.

*Member of the Board of Governors Crop Protection Institute* (three years), W. H. Martin, New Jersey Agricultural Experiment Station, New Brunswick, N. J.

*Representative on Editorial Board, American Journal of Botany,* G. W. Keitt, University of Wisconsin, Madison, Wisconsin.

The following temporary committees were appointed to serve throughout the meeting:

*Auditing Committee,* M. C. Goldsworthy and R. D. Rands.

*Committee on Elections,* C. E. F. Guterman and H. N. Brooks.

*Committee on Resolutions,* Annie R. Gravatt, Max W. Gardner, and G. R. Bisby.

## REPORT OF THE SECRETARY-TREASURER, 1934

During the year 1933 the net loss in membership in the Society was 53, but for the year 1934 I am glad to be able to report an increase. We have had fewer resignations, fewer suspensions for non-payment, and more reinstatements of former members and applications from new members. We started the year with 774 members. We lost only 36 this year as compared with 84 in 1933. Two of our members died, 10 resigned and 24 were suspended for non-payment of dues. Seventeen former members were reinstated. The election of 43 new members at this meeting brings the total mem-

bership to 798, a net gain of 24. Of the total number, 102 are paid up life members and 68 are life-sustaining.

In this, my sixth and last report as Secretary-Treasurer and Business Manager, I wish to acknowledge the able assistance rendered during the first two years by Miss Mary G. Van Meter, of the Plant Disease Survey, U. S. Department of Agriculture, and during the last four years by my wife. With the increased time required for official duties, the active interest of Miss Van Meter and Mrs. Meier in the welfare of the Society, and their assistance with the work, was indispensable.

STATEMENT OF ACCOUNTS FOR THE YEAR ENDING NOVEMBER 30, 1934

*Receipts*:

| | | | |
|---|---|---|---|
| Balance from 1933 | | | $1,782.00 |
| Annual dues: | | | |
| 1931 | $5.00 | | |
| 1932 | 35.00 ($20.00 life) | | |
| 1933 | 121.53 ( 39.83 life) | | |
| 1934 | 2,299.29 (339.35 life) | | |
| 1935 | 1,208.93 (270.04 life) | | |
| 1936 | 26.50 ( 10.00 life) | $3,696.25 | |
| Donations to Lyman Fund included in checks for dues | | 4.00 | |
| Sales included in checks for dues | | 1.60 | |
| Checks to replace those returned by bank | | 10.00 | |
| Total receipts | | | 3,711.85 |
| | | | $5,493.85 |

*Expenditures*:

| | |
|---|---|
| Member subscriptions transferred to PHYTOPATHOLOGY (1934, part 1931, 1932, 1933) | $2,870.42 |
| Transferred to Sinking Fund Savings Account | 400.00 |
| Secretarial work | 212.87 |
| Printing (programs, ballots, bills, etc.) | 75.80 |
| Postage and stamped envelopes | 41.36 |
| Telephone and telegraph | 8.81 |
| Checks returned by bank and charge for collection of check | 10.25 |
| Expenses of Secretary-Treasurer, Boston meeting | 46.35 |
| Expenses of President, Boston meeting | 66.20 |
| Expenses of representative, National Research Council meeting | 46.90 |
| Expenses of Secretary-Treasurer, advance trip to Pittsburgh | 3.65 |
| Contribution for Biologists' Smoker, Boston meeting | 15.00 |
| A. A. A. S. registration fee for Journal Exhibit | 1.00 |
| Donations transferred to Lyman Fund | 4.00 |
| Sales transferred to PHYTOPATHOLOGY | 1.60 |
| Supplies | 1.90 |
| Federal tax on checks | 0.58 |
| Total expenditures | 3,806.69 |
| Balance on hand | 1,687.16 |
| | $5,493.85 |

SINKING FUND

The Sinking Fund, obtained by deducting $5.00 (formerly $6.00) from each life-sustaining membership payment, now stands at $8,321.00, of which $5,000 is invested in first mortgage notes. $3,181.00 is deposited in a savings account with the Riggs National Bank of Washington, D. C. The remainder, $140.00, has not yet been transferred from the checking account of the Society. The income from this fund now amounts to about $385.00 and is used to help defray the cost of publishing PHYTOPATHOLOGY.

Respectfully submitted,
F. C. MEIER, *Secretary-Treasurer.*

REPORT OF THE BUSINESS MANAGER OF PHYTOPATHOLOGY FOR 1934

STATEMENT OF ACCOUNTS FOR THE YEAR ENDING DECEMBER 4, 1934

| | | | |
|---|---|---|---|
| *Receipts*: | | | |
| Balance from 1933 | | | $1,067.64 |
| Subscriptions: 1933 | $ 63.55 | | |
| 1934 | 2,838.36 | | |
| 1935 | 252.17 | | |
| 1936 | 1.00 | $3,155.08 | |
| Member subscriptions 1933 and earlier | | 237.30 | |
| Member subscriptions 1934 | | 2,633.12 | |
| Sales of back numbers | | 312.98 | |
| Special sale of 1933 | | 84.00 | |
| Advertising: 1933 | $ 93.58 | | |
| 1934 | 722.44 | 816.02 | |
| Interest on 1st mortgage notes | | 299.50 | |
| Interest on Sinking Fund Savings Account | | 119.69 | |
| Grant from National Academy of Science | | 750.00 | |
| From University of California for Esau illustrations | | 70.76 | |
| Total receipts | | | 8,478.45 |
| | | | $9,546.09 |
| *Expenditures*: | | | |
| Printing and Distributing PHYTOPATHOLOGY: | | | |
| Vol. XXIII, No. 11 | $ 502.48 | | |
| Vol. XXIII, No. 12 | 415.79 | | |
| Index to Vol. XXIII | 180.17 | $1,098.44 | |
| Vol. XXIV, No. 1 | 504.01 | | |
| No. 2 | 503.48 | | |
| No. 3 | 781.19 | | |
| No. 4 | 697.29 | | |
| No. 5 | 629.74 | | |
| No. 6 | 689.62 | | |
| No. 7 | 745.50 | | |
| No. 8 | 493.86 | | |
| No. 9 | 400.96 | | |
| No. 10 | 374.09 | 5,819.74 | |
| Postage | | 607.77 | $7,525.95 |

| | | |
|---|---|---|
| Secretarial work for Business Manager | 221.87 | |
| Secretarial work for Editor in Chief | 258.50 | |
| Miscellaneous expenses of office of Editor in Chief | 43.20 | |
| Secretarial work for Advertising Manager | 41.34 | |
| Commission and expenses of Advertising Manager | 74.02 | |
| Stamps and envelopes | 49.24 | |
| Telephone and telegraph | 3.24 | |
| Subscription refunded | 6.00 | |
| Preprints of abstracts for Boston meeting | 21.59 | |
| Insurance on back volumes stored at Univ. of Maryland | 46.71 | |
| Supplies and shipping material to Boston | 1.40 | |
| Printing | 5.25 | |
| Federal tax on checks | 0.96 | |
| Total expenditures | | $8,299.27 |
| Balance on hand | | 1,246.82 |
| | | $9,546.09 |

At the end of 1933 there were 502 non-member subscribers to PHYTOPATHOLOGY including 7 complimentary subscriptions. During the year there were 28 cancellations, and 11 suspensions due to non-payment. With the addition of 78 new subscribers, the net gain was 39, increasing the list to 541.

Respectfully submitted,
F. C. MEIER, *Business Manager.*

REPORT OF THE EDITOR IN CHIEF

With the closing of the current year, it is well to take stock of our Society's accomplishment as registered within the pages of our Journal PHYTOPATHOLOGY. As an indicator of research trends and progress in plant pathology, it is fairly reliable and, on the whole, a periodical in which the Society is fully justified in entertaining genuine pride, thanks to the continued improvement in the quality of the contributions submitted. On the other hand, we should be constantly aware of the fact that the need for continued improvement prevails.

Reports coming to us from home and abroad remind us that PHYTOPATHOLOGY is one of the best and most valuable journals of its kind in the world. Occasionally and too often, however, we receive complaints that, in brief, read or sound like the following: Too many of the papers are longer than is warranted by their essential substance; some of the contributions are of doubtful timeliness; not enough thought is given to selection of illustrative material; tabular matter, in many instances, should be better organized and condensed; manuscripts should be critically read by more people qualified to pass upon their merits; and so on, indicating that in the judgment of some of our readers, there still is room for further improvement.

The 24th volume of PHYTOPATHOLOGY contains 1386 pages of printed text and illustrations, or 380 pages more than appeared in Volume 23. The 1,386 pages of our journal for 1934 are classified as follows: Ninety-one articles, 58 phytopathological notes, 4 reports of regional or other meetings, 9 book reviews, 122 abstracts (4 by title only), and 263 text figures, and 3 plates. During the period January 1 to December 31, inclusive, approximately 153 manuscripts of articles, phytopathological notes, reports, book reviews, etc., and 175 abstracts (4 by title only) were submitted. Of this number 10 major manuscripts were returned to their authors for revision, 1 was found unaccept-

able, and 3 were withdrawn by authors. Of the manuscripts submitted in 1933, but too late for publication that year, 1 was withdrawn by the author and 2 were found unacceptable. In addition to the several papers published in 1934, 28 articles, 15 phytopathological notes, one book review, and 110 abstracts (1 by title only) are now (December 31) in press. The index for Volume 23 was published as a supplement to the April, 1934, number of PHYTOPATHOLOGY.

*Summarized Report for the Triennium* 1932 *to* 1934. The 22nd, 23rd, and 24th volumes of PHYTOPATHOLOGY comprise 244 articles, 124 phytopathological notes, 9 reports, 21 book reviews, 407 abstracts, 635 text figures, and 4 plates. The foregoing items comprised a total of 3394 printed pages, which, at an estimated average of $6.00 per page, cost the Society and a limited number of paying contributors, or supporting institutions, $20,364 for the triennium.

Of the total number of papers received, 31, or approximately 8 per cent, were returned to authors for revision; 16, or approximately 4 per cent, were found unacceptable and 8, or approximately 2 per cent, were voluntarily withdrawn.

The author wishes here to make grateful acknowledgment of the helpful service unselfishly rendered by the several members of the Editorial board, by Miss Frances W. Todd, and by the Science Press Printing Company.

Respectfully submitted,

H. B. HUMPHREY, *Editor in Chief.*

REPORT FOR PHYTOPATHOLOGICAL CLASSICS

FROM DECEMBER 8, 1933, TO DECEMBER 15, 1934

| | | |
|---|---|---|
| Total number of Classic No. 1 on hand | | 451 |
| Number of Classic No. 1, sold, | 58 | |
| Number of gratis for advertising purposes | 3 | |
| Unaccounted for | 1 | |
| Total number disposed of | | 62 |
| Balance on hand | | 389 |
| Total number of Classic No. 2 on hand | | 656 |
| Number of Classic No. 2 sold | 63 | |
| Number gratis for advertising purposes | 3 | |
| Unaccounted for | 2 | |
| Total number disposed of | | 68 |
| Balance on hand | | 588 |
| Total number of Classic No. 3 on hand | | 1020 |
| Number of Classic No. 3 sold | 216 | |
| Number gratis for advertising purposes | 3 | |
| Number complimentary copies | 9 | |
| Unaccounted for | 3 | |
| Total number disposed of | | 228 |
| Balance on hand | | 792 |

Classics sold as follows:

| | | | |
|---|---|---|---|
| Classic No. 2 | 1 copy @ $0.50 | | $0.50 |
| Classic No. 3 | 155 copies @ 0.50 | | 77.50 |

| | | | | |
|---|---|---|---|---|
| Classic No. 1 & 2 | 2 copies | @ | 0.75 | 1.50 |
| Classic No. 2 & 3 | 5 copies | @ | 1.00 | 5.00 |
| Classic No. 1 & 3 | 1 copy | @ | 0.75 | 0.75 |
| Classic No. 1, 2, 3 | 52 copies | @ | 1.25 | 65.00 |
| Classic No. 1, 2, 3 | 2 copies | @ | 1.50 | 3.00 |
| Classic No. 1, 2, 3 | 1 copy | @ | 2.00 | 2.00 |
| Total | | | | $155.25 |
| Due on account | | | | 4.75 |
| | | | | $150.50 |
| Plus Foreign Exchange | | | | 0.23 |
| Total receipts for the current year | | | | $150.73 |
| Balance from last year | | | | 188.25 |
| Total | | | | $338.98 |
| Expenditures: | | | | |
| Printing of Classic No. 3 | | | | $121.40 |
| Postage | | | | 10.07 |
| Total expenditures | | | | $131.47 |
| Balance on hand December 15, 1934 | | | | $207.51 |

Respectfully submitted,
H. H. WHETZEL, *Business Manager.*

REPORT OF THE ADVERTISING MANAGER

During 1934 a total of 155 advertisements were run in PHYTOPATHOLOGY. This advertising consisted of a total of 108 pages. Of these, 50 were non-revenue, among which were exchange ads with other journals, meeting announcements and advertisements for Phytopathological Classics. The gross amount contracted for rose from $561.50 in 1933 to $1,000.45 in 1934, an increase of about 80 per cent. This upward trend is encouraging.

Respectfully submitted,
R. S. KIRBY, *Advertising Manager.*

REPORT OF COMMITTEE ON FOREIGN PLANT DISEASES

Your Committee submits for consideration the following report:

The Committee believes that The American Phytopathological Society, composed largely of men in public service and constituting a representative organization capable of forming intelligent opinion on the biological aspects of plant quarantine and restrictive measures, owes as a distinct duty to the nation to aid in the solution of the problems of guarding this country from foreign plant diseases. We recommend that the Council consider the advisability of scheduling a special session on quarantine problems at the next Annual Meeting. Among the numerous phases of the subject, the following appear to the Committee to be urgent and should be discussed at this or subsequent meetings:

1. The advisability of establishing a more adequate detention service through which questionable introductions may be safely held under observation.
2. The desirability of arranging for the testing of native plants under foreign

conditions to determine their reaction to foreign diseases not yet introduced here.

3. Your committee recalls a recommendation of December, 1927, on the desirability of establishing specialists in foreign countries to obtain specific information on foreign diseases, and strongly urges that, wherever possible, this policy be adopted by the administration.
4. The attitude that should be taken in regard to the exclusion of biological races of parasites already present in this country that might complicate the efforts here in breeding resistant plants or introduce more virulent parasitic types.
5. The safety measures that are needed in connection with the introduction of living cultures or viable specimens of parasites from abroad.

The American Phytopathological Society urges that in view of the serious situation presented by the Dutch elm disease special efforts should be made by state and federal authorities to extend search for the disease to all territory where the elms are of importance.

The Society further recommends that careful consideration should be given by the federal government and the states concerned to the feasibility of carrying out an eradication program against potato wart, as an alternative to continuing present quarantine measures or allowing the disease to spread.

Respectfully submitted,

C. R. ORTON, *Chairman*,

(See page 538 for Society action on this report.)

### COMMITTEE ON NECROLOGY

During the calendar year 1934, there have been two deaths as follows:

Dr. Frank Lincoln Stevens, August 16, 1934.

Mr. Herman J. Ninman, October 24, 1934.

A. G. JOHNSON, *Chairman*,
G. P. CLINTON,
M. B. WAITE.

### REPORTS OF OTHER COMMITTEES

*Committee on Elections.* The committee on elections, after having opened sealed-ballot envelopes and counting votes for each nominee, reports election of the following officers: President, H. T. Güssow; Vice-President, F. C. Meier; Secretary, H. P. Barss; Councilor, J. C. Walker.

C. E. F. GUTERMAN, *Chairman*,
H. N. BROOKS.

*Auditing Committee.* We have examined the books of the Secretary-Treasurer of the Society. In view of the fact that the above office is to be vacated, by the incumbent, at the end of the present year, a minute examination was conducted. We beg to advise the Society that we have found the accounts of the Secretary-Treasurer correct in all their details and that the books are in a most excellent order.

M. C. GOLDSWORTHY,
R. D. RANDS.

### REPORT OF REPRESENTATIVES ON BOARD OF GOVERNORS, CROP PROTECTION INSTITUTE

During the year 1934 there were conducted 19 projects on part- or full-time basis. Of this total 7 were concerned directly with problems in plant pathology. All projects

were placed according to facilities and areas most adaptable for conducting the research and, where possible, conveniently accessible for the supporting company. The projects had headquarters or work conducted in the following states: New Hampshire, Illinois, Delaware, New Jersey, Massachusetts, Michigan, Iowa, Ohio, California, Connecticut, Florida, Virginia, Pennsylvania, Indiana, Missouri, and Washington. There were 5 publications issued in the Crop Protection Institute bulletin series. During the year 1934 the following representatives served as the Board of Governors: W. C. O'Kane, Chairman, Durham, New Hampshire; W. P. Flint, Illinois; Charles H. Richardson, Ames, Iowa; J. F. Adams, Vice-Chairman, Newark, Delaware; W. H. Martin, New Brunswick, New Jersey; I. E. Melhus, Ames, Iowa; H. J. Patterson, College Park, Maryland; W. H. MacIntire, Knoxville, Tennessee; E. D. Ball, Tuscon, Arizona.

The following summary presents the research and development projects directed by the Crop Protection Institute for the calendar year 1934:

*Copper in Plant Nutrition.* A study of increased yields brought about through the addition of copper salts to soils, including various typical soils not deficient in copper, and including a considerable number of important staple crops. Increased yields of economic importance have been secured. This project is financed by the Nichols Copper Company in cooperation with the Copper and Brass Research Association. Headquarters at the Delaware Experiment Station, but additional work carried on in various other States. Investigators employed include Raymond Russell, J. D. Hartman, W. L. Churchman and Woodrow Singles.

*New Copper Fungicides.* A project intended to develop new and better copper compounds that may supplement or take the place of those now in use. At least one new compound of unusual properties has been developed. This work financed by the Nichols Copper Company. Headquarters at the Delaware Experiment Station. Alexander Nikitin employed as investigator.

*Oil Sprays.* A continuation of a project in which improvements in the composition of oil sprays for summer application have been the objective. Promising results have been secured. This project financed by the Standard Oil Co. of Indiana. Headquarters at the Illinois Experiment Station. Investigator employed on the project, John L. Alsterlund.

*Pyrethrum Culture in the United States.* A continuation of a thorough and extensive study intended to solve the various problems involved in commercial growing of pyrethrum in the continental United States. Good progress has been made and a solution has been arrived at for various problems involved in the domestic production of this important plant. This project supported by Stanco Incorporated. Headquarters at the New Jersey Experiment Station, with extensive supplemental work in Pennsylvania. Investigator employed, R. E. Culbertson.

*Preliminary Study of Organic Compounds of Promise as Insecticides and Fungicides.* An exploration of various organic compounds which may be of value in the development of new and better sprays. Supported by the Monsanto Chemical Company on a part-time basis. Headquarters at the New Hampshire Experiment Station. Investigator employed, W. A. Westgate.

*Improvement in Pyrethrum Sprays.* Continuation of a project designed to develop more efficient ways of using the active principles of pyrethrum flowers. A new and improved method of using the principles has been discovered. This work financed by J. C. Makepeace. Headquarters at Wareham, Mass., with supplemental work in Florida and New Hampshire. Investigator employed, George L. Walker.

*Development of a New Spray for Codling Moth and Other Insects.* An investigation of a considerable series of new organic compounds among which several have been discovered which are giving promising results and one in particular which has reached

the point of successful field use. This work financed by the Dow Chemical Company. Headquarters at the Iowa Experiment Station, with supplemental work in Michigan and further work in various other States. Investigator employed, John F. Kagy.

*New Fumigants.* Continuation of a project utilizing carbon dioxide in conjunction with other materials for the fumigation of stored products. A new principle in utilization of carbon dioxide has been discovered, and a new mixture of gases developed which is now on the market under the name Proxate. The fumigant is safe for the operator, is not explosive, is inexpensive, and is giving consistently satisfactory results. Headquarters at the Iowa Experiment Station and in Chicago. Investigator employed, R. M. Jones.

*New Fungicides and Insecticides.* A study of a long series of organic compounds that has resulted in the development of at least one material of promise. This project suspended at present awaiting manufacturing facilities. Supported by the National Aniline Company. Headquarters at the Delaware Experiment Station. Investigator employed, C. N. Priode.

*New Contact Insecticide.* An investigation intended to develop a means of utilizing Halowax oil as the active component of a new contact insecticide and resulting in the invention of a new material which is giving excellent results and has advanced to semi-commercial use. Supported by the Halowax Corporation. Headquarters at Ohio State University. Investigator employed, E. P. Breakey.

*Sulphuric Acid in Weed Control.* A study planned to disclose satisfactory methods of utilizing dilute sulphuric acid in control of weeds in cereals, notably mustard in barley, together with means of utilizing concentrated sulphuric acid in combination with other substances for eradication of resistant weeds. Machinery for application of dilute sulphuric acid has been devised and results of economic importance have been secured. This investigation financed by the Freeport Sulphur Company. Headquarters at the Davis Substation of the University of California. Investigator employed, Willis E. Ball.

*Development of New Insecticides and Fungicides Utilizing Sulphur and Carbon Disulphide.* A thorough study of the possibilities of these two substances, both alone and in combination, and both from the insecticidal and from the fungicidal point of view. Financed by the Stauffer Chemical Company. Headquarters at the Connecticut Experiment Station and the Massachusetts Experiment Station. Investigators employed, M. V. Anthony, and T. T. Ayers.

*Plant Extracts, Including Pyrethrum, Derris, and Others.* A project planned to develop better means of utilizing the active principles of various plant extracts in insect control. Supported by S. B. Penick & Company. Headquarters at the New Hampshire Experiment Station and in Southern States. Investigators employed, O. J. Smith and W. C. Baker.

*Cattle Sprays.* A study of the composition of cattle sprays and their improvement. Financed by the Hercules Powder Company. Headquarters at the Delaware Experiment Station. Investigator employed, A. M. Pearson.

*New Spray Materials and New Means for Their Application.* A project planned to develop improved methods of application of spray materials in greenhouses, home gardens, and similar situations, together with suitable materials for the new device. Supported by the Mergenthaler Linotype Company. Headquarters at the new New Hampshire Experiment Station. Investigator employed, William H. O'Kane.

*Codling Moth Sprays.* A project for the development of new and safe spray materials for codling moth control. One or more materials of promise have been discovered. Headquarters at the Delaware and Indiana Experiment Stations, with sup-

plemental work in Virginia, Missouri, and Washington. Supported by the General Chemical Company. Investigator employed, Merrill M. Darley.

Respectfully submitted,

J. F. ADAMS,
I. E. MELHUS,
W. H. MARTIN.

*Report of the Committee on Resolutions.* The American Phytopathological Society wishes to thank the American Association for the Advancement of Science for making general arrangements for these meetings. It is also grateful to Prof. O. E. Jennings of the University of Pittsburgh and his associates for arranging many details.

To the management of the Hotel William Penn The American Phytopathological Society wishes to express its appreciation of the many courtesies extended during these meetings. Special thanks are due Mr. Gerald P. O'Neill, General Manager, and Mr. Sam K. Smith, Sales Manager.

Applause and appreciation are due R. S. Kirby, A. L. Pierstorff, F. J. Schneiderhan, and W. H. Thurston, the committee that arranged the entertainment at the annual dinner. Delightful interludes were afforded by the accordian and vocal solos of Miss Leon Metzger.

Sincere appreciation is extended to F. C. Meier, Secretary-Treasurer of the Society and Business Manager of PHYTOPATHOLOGY for six years of efficient and unceasing service, and to H. B. Humphrey for his continued efficient editorship of PHYTOPATHOLOGY.

ANNIE R. GRAVATT,
MAX W. GARDNER,
G. R. BISBY.

### ACTION OF THE COUNCIL

In addition to making the appointments of the officers mentioned earlier in this report, the Council submitted the following actions, which were approved by the Society:

1. It is recommended that the Editor in Chief of PHYTOPATHOLOGY be authorized to recommend to the Council the necessary associate editors for 1935.
2. It is recommended that the Editor in Chief of PHYTOPATHOLOGY be allowed actual expenses up to or within $350.00 for secretarial and editorial assistance during 1935.
3. It is recommended that the incoming Secretary prepare a revised membership list and revised constitution, and that the Treasurer and Business Manager be authorized to pay for printing these.
4. It is recommended that the Secretary-Treasurer be authorized to pay part expense ($49.50) incurred by our representative in travelling to the 1934 meeting of the National Research Council.

### CONSTITUTION AMENDED

In accordance with provisions of Article X, the Society voted to amend the Constitution by discontinuing the office of Secretary-Treasurer, and establishing the separate offices of Secretary and Treasurer, each with a three-year term. Under Standing Rules it was voted to increase the term of office for the Business Manager of PHYTOPATHOLOGY from 1 to 3 years.

### OTHER BUSINESS

The reports of officers, committees, and representatives as printed above were accepted. Action of the Council as indicated above was approved by the Society.

At the request of the Council, Dr. Donald Reddick reported in detail on the steps taken and progress made during the past five years toward the formation of some type of international organization of plant pathologists. In line with previous resolutions of the Society, the Council was authorized to effect a union with the Phytopathological Section of the International Union of Biological Societies, at such time and in such manner as may be practicable.

Reading of the report of the Committee on Foreign Plant Diseases was followed by a motion, made by Dr. E. C. Stakman, to amend the report as it relates to the Dutch elm disease as follows:

The Society also wishes to commend the Department of Agriculture and the cooperating States for the efforts now being made to investigate the disease and control or eradicate it. The Society wishes to urge, furthermore, that the Department do everything in its power to learn all of the pertinent facts in connection with the disease and to make every reasonable attempt to control or eradicate it.

After discussion of the amendment and the need for an active program for control of the Dutch elm disease, the amendment was passed and the chairman of the committee was instructed to present in person the Society's action relating to the Dutch elm disease and the potato-wart disease to the Secretary of Agriculture, the Chief of the Bureau of Plant Industry, and the Chief of the Bureau of Entomology and Plant Quarantine. (This was done by C. R. Orton on January 29, 1935).

A motion made by Charles Chupp that the Council reorganize and expand the membership of the Committee on Extension to include research representation was carried. The Society also passed a motion to devote a session at the next annual meeting to a discussion of the coordination of research and extension.